Exhibit 109

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METHODOLOGY FOR THE MEASUREMENT OF AIRBORNE ASBESTOS BY ELECTRON MICROSCOPY

by

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FOREWORD

PREFACE

ABSTRACT

The provisional electron microscope methodology for measuring the concentration of airborne asbestos fibers was refined. The methodology is divided into separate protocols. The step-by-step procedures for each protocol are nearly identical, so that cumulative data can be obtained and uncertainties, especially in asbestos identification, can be clarified. The operational steps encompass (1) type of sample, (2) collection and transport, (3) sample preparation, (4) examination under the transmission electron microscope (TEM) and data collection, (5) data reduction and reporting of results, and (6) quality control-quality assurance.

The TEM analytical protocol is subdivided into three levels of analysis: Level I, for screening many samples; Level II, for regulatory action; and Level III, for confirmatory analysis of controversial samples. Because identification of asbestos structures is critical, the level of analysis is directly related to the information sought:

Level I--morphology and visual selected area electron diffraction (SAED) pattern recognition.

Level II--morphology; visual SAED; and elemental analysis.

Level III--morphology; visual SAED; a selected number of SAED micrographs of zone-axis patterns; and elemental analysis.

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CONTENTS

CONTENTS (continued)

CONTENTS (continued)

TABLES

FIGURES

LIST OF ABBREVIATIONS

AEM	analytical electron microscope
EDS	energy dispersive spectrometer
EM	electron microscope
JCPDS	Joint Committee on Powder Diffraction Standards
LTA	low-temperature ashing
NIOSH	National Institute of Occupational Safety and Health
PLM	polarized light microscopy
QC/QA	quality control/quality assurance
SAED	selected area electron diffraction
SEM	scanning electron microscope
STEM	scanning transmission electron microscope
TEM	transmission electron microscope
TSP	total suspended particulates
UICC	Union Internationale Contre le Cancer
XRD	x-ray diffraction
XRF	x-ray fluorescence

SECTION 1

INTRODUCTION

Asbestos is recognized as a health hazard, especially if inspired into the alveolar region of the respiratory tract. Asbestos may be present in air samples, water samples, biological or clinical samples, and other miscellaneous bulk samples, such as ores and food. These various types of samples require different collection methodologies and diverse preparation techniques.

Asbestos analysis methodologies may be categorized as bulk-material analyses, or those providing concentration information, and single-fiber analyses, or those providing morphology, size distribution, and concentration. Bulk-material analysis techniques, which include infrared spectroscopy, differential thermal analysis, and x-ray diffraction analysis (XRD), are limited by an inability to analyze concentrations of less than 1 μg, and by an inability to differentiate between fibrous and nonfibrous forms of minerals.

Single-fiber analysis techniques include optical microscopy and electron microscopy. Optical microscopy employing phase contrast has been promulgated into a monitoring method for the workplace environment (NIOSH-P&CAM 239). In addition, promulgation of a monitoring method for bulk-material asbestos samples (building insulation) using polarized light microscopy (PLM) is presently being considered. However, optical microscopic techniques cannot determine fibers of less than approximately 1 μm in diameter, and phase contrast cannot differentiate between asbestos and nonasbestos fibers.

The electron microscope (EM) provides particle morphology and size, and a degree of identification. A comprehensive study of various EM procedures (Samudra et al., 1977) was conducted in development of a provisional methodology manual, Electron Microscope Measurement of Airborne Asbestos Concentrations (Samudra, 1978). Three EM methods are available: the scanning electron microscope (SEM), the transmission electron microscope (TEM), and the analytical electron microscope (AEM). The SEM, with an x-ray energy-dispersive spectrometer (EDS), permits visual characterization (analogous to reflection optical microscopy) and fiber identification by elemental analysis. The TEM, providing an increased data-acquisition capability, permits visual characterization (in the transmitted mode) and fiber identification by crystal structure analysis. The AEM is a TEM with an EDS, and with the added capability of SEM/STEM (scanning transmission electron microscope) operation, which permits visual characterization (morphology and size) as well as fiber identification using both crystal structure by selected area electron diffraction (SAED) and elemental analysis by EDS.

The original EM methodology was developed for the U.S. Environmental Protection Agency (EPA) for measuring airborne asbestos concentrations, specifically for ambient air and for use as a "screening" tool. Development guidelines included attainable precision and accuracy of results; relative rapidness in use; cost-effectiveness; applicability to a large number of laboratories possessing a TEM (at that time, very few laboratories had TEM's with x-ray analysis capability or an AEM); and procedural steps to be independent of unique or exceptional in-house capabilities of a single laboratory (that is, interlaboratory precision rather than intralaboratory).

In usage, the EM method was successful within its prescribed limitations--that is, the precision and accuracy of results between laboratories using the complete method was good. However, problems that had been recognized in the study developing the methodology (Samudra et al., 1977) arose in the areas of (1) interpretation of airborne, (2) sample collection, (3) need for more exacting identification of asbestos, especially of amphibole type, and (4) use of only part of the methodology.

The present study was undertaken to refine the methodology. The problem areas and related criticisms were addressed within the underlying goals and guidelines set for optimizing the methodology. Protocols similar to a cookbook were not possible since basic knowledge or training was required regarding (1) sample collection, (2) preparation of samples for EM, (3) use of the TEM-AEM, and (4) diffraction pattern analysis. The refined methodology is based on an assumption that each intended user of a particular level of analysis has the necessary background and training to use it.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

The EM methodology for measuring the concentration of airborne asbestos fibers has been refined and specified, and is recommended for field evaluation. The methodology is based on a TEM analytical protocol that is divided into three levels of effort: Level I, for screening many samples; Level II, for regulatory action; and Level III, for confirmatory analysis of controversial samples. The three-level analytical methodology is cost-effective, and will provide the required results for proper assessment of asbestos.

SECTION 3

GUIDELINES FOR UNDERSTANDING THE METHODOLOGY

The methodology is divided into separate protocols. The step-by-step procedures for each protocol are nearly identical, so that cumulative data can be obtained and uncertainties, especially in asbestos identification, can be clarified. These operational steps are:

(1) Type of Sample--Source

(2) Sample Collection and Transport

(3) Sample Preparation for Analysis--Grid Transfer

(4) TEM Examination and Data Collection

(5) Data Reduction and Reporting of Results

(6) Quality Control/Quality Assurance (QC/QA).

The analytical protocol under the TEM examination and data collection procedure is subdivided into three levels of increasing analytical effort in terms of requiring an instrument of greater capability, an electron microscopist with greater expertise, and a longer analytical time. Level I, a monitoring or screening methodology, resembles the present EPA provisional methodology (Samudra et al., 1978; Anderson and Long, 1980). Level II is a regulatory method requiring additional analytical criteria to establish asbestos identification limits, and to provide guidance for Level I analyses by confirming or clarifying visual SAED patterns. Level III, the most sophisticated and the costliest of the methods, is intended for confirming asbestos identification, especially in judicial controversies and other special situations.

In Sections 4, 5, and 6, the protocols for each of the three levels of analysis are presented independently of each other, and thus procedures common to each are repeated. All figures are presented in Appendix A.

Section 7 describes modifications for using the methodology on archival samples, which are samples collected on nonprocedural filter substrates, or samples collected without regard to filter loading levels. Section 8 describes analysis of inorganic sources in bulk-air samples or in bulk form. Section 9 concludes the report with a discussion of analytical aids pertaining to the limits of detection, preparation of blanks, use of computers, magnification calibration, and statistical methodology.

General guidelines for understanding the methodology are discussed in the following paragraphs.

LEVEL OF ANALYSIS

Knowledge of the history, source, and location of the sample, and the purpose and objective of the analysis aids in selecting the correct level of analytical effort. Simply "grinding the samples out" neither is cost-effective nor produces the best results, especially for Level II and Level III analyses. Instead of all Level I, all Level II, or all Level III, the majority of the analyses may be Level I, followed by some Level II. Level III could be used in its entirety or only at the analytical phase. If the source is known to contain no amphibole-type interference, or if chrysotile is of interest, gold-coating can be eliminated.

If a legal proceeding is anticipated, Level III analysis will be required where a chain-of-custody record is kept from collection, transport to the laboratory, preparation, analysis, data reduction, and reporting of results. EM finder grids must be used for grid transfer. In addition, for quality assurance, a second laboratory must be available for analyzing a portion of the sample using the same degree of custodial care. QC/QA protocols must be observed and records kept.

Whenever possible, and especially for unknown source samples, 10 to 20% of each set of samples should be analyzed by Level II analysis prior to using Level I as a screening procedure.

Level I is a relatively rapid procedure, and can be used by many laboratories with access to a conventional TEM. However, Level I results should not be used in legal proceedings. If "positives" or "false positives" are found, especially in areas where asbestos is known to be absent, and the field blank and laboratory blank have been checked, Level II analysis, and possibly Level III analysis, should be performed.

ORDER OF ANALYSIS

The order of analysis is (1) field blanks, (2) laboratory blanks (if needed), and (3) field samples.

COLLECTION AND REPORTING

The counting rule, "minimum 100 fibrous structures per known area (complete grid opening) or 10 grid openings, whichever is first," is a minimum rule for cost limitation. For very low asbestos presence, or for asbestos contamination studies, where particulate loading is high and asbestos presence very low, counting 20 grid openings from each of 2 grids (10 per grid) is recommended.

The EM magnification factor is very high or, conversely, the area of deposit examined is very small. Therefore, although the electron microscopist may report a zero count, the notation "Below Detectable Level" is more appropriate in the sample report. Along the same lines, the electron microscopist

should report observations, measurements, and conclusions as objectively as possible, realizing the subjective nature of his decision-making, such as parallel-sided, 3:1 aspect ratio, number count, size measurements, recognition-discrimination of SAED patterns, and categorizing of asbestos structure.

Data reduction and reporting of results must be consistent and stated. Dimensions of X-fibers (unknown length since complete fiber is not visible) may be doubled, not counted at all, or presented separately. Doubling of the visible portion is recommended, and should be so stated in the report.

Mass or conversion of size measurements to an assumed shape-volume-density relationship, is calculated, and thus is the least reliable of the data, especially for X-fibers, bundles, clusters, and matrices.

Although morphology, SAED, and XRF either singly or in combination will provide identification of asbestos, not all structures will be identified. The nature of the asbestos structure prevents analysis of all structures by SAED and/or by elemental analysis with EDS. Such factors as specimen thickness, orientation, and proximity to other particulates or to the grid wire will prevent attainment of good SAED patterns and limit the effectiveness of chemical analysis.

COSTS

Levels I, II, and III analyses are estimated to require 200, 400, and 1200 min per analysis, respectively. Additional costs will result from collection, preparation, and reporting of results. The equivalent monetary costs will depend on the laboratory rates of the personnel involved.

APPLICATION TO NONAIRBORNE SOURCES

Although the methodology has been developed for airborne asbestos, other types of samples from different sources can be analyzed if the samples are finely divided and placed with proper loading and uniform distribution either on a polycarbonate membrane filter or on a carbon-coated EM grid. Of course, the limitations of the collection and preparation steps must be known and accounted for to prevent inaccuracies in comparing results.

GEOGRAPHICAL CONSIDERATIONS

In some parts of the country, such as the Upper Great Lakes area, the possibility of misidentification is much greater because some nonamphibole minerals have visual SAED patterns that closely resemble those of amphiboles. Gold-coating and Level II analysis will help in differentiating between these minerals.

LABORATORY CONDITIONS

Asbestos analysis involves sustained microscopy for periods of 3 to 7 hours with unscheduled rest breaks. Subjective decisions regarding such factors as morphology, size measurement, visual identification, and possible

EDS make it difficult to break a manipulative physical routine or rhythm. Therefore, a professional environment for the microscopist is essential for effective asbestos analysis. In particular, such factors as unnecessary or redundant procedural steps, lack of personal recognition, and unreasonable deadlines may contribute to poor precision.

SECTION 4

LEVEL I ANALYSIS

SUMMARY OF PROTOCOL

Level I analysis is a monitoring or screening technique. It assesses the amount and type of asbestos structures in the atmosphere through the following steps:

(1) A known volume of air is passed through a polycarbonate membrane filter (pore diameter, 0.4 μm; filter diameter, 37 or 47 mm) to obtain approximately 5 to 10 μg of particulates per cm^2 of filter surface.

(2) The particulate-laden filter is transported in its own filter holder.

(3) The filter is carbon-coated in the holder.

(4) The particulates are transferred to an EM grid using a refined Jaffe wick washer.

(5) The EM grid, containing the particulates, is gold-coated lightly.

(6) The EM grid is examined under low magnification (250X to 1000X) followed by high-magnification (16,000X on the fluorescent screen) search and analysis.

(7) A known area (measured grid opening) is scanned, and the fibrous structures (fibers, bundles, clusters, and matrices) are counted, sized, and identified as to asbestos type (chrysotile, amphibole, ambiguous, or no identity) by morphology and by observing the SAED pattern.

(8) The observations are recorded--a minimum of 100 fibrous structures or 10 grid openings, whichever is first.

(9) The data are reduced and the results reported.

EQUIPMENT, FACILITIES, AND SUPPLIES

The following items are required for Level I analysis:

(1) An 80 or 100-kV TEM with a fluorescent viewing screen inscribed with graduations for estimating the length and width of fibrous particulates.

(2) A vacuum evaporator with a turntable for rotating specimens during coating, for such uses as carbon-coating polycarbonate filters, gold-coating EM grids, and preparing carbon-coated EM grids.

(3) An EM preparation room adjacent to the room housing the EM. This room should either be a clean-room facility, or contain a laminar-flow class-100 clean bench to minimize contamination during EM grid preparation. Filter handling and transfer to EM grids should be performed in a clean atmosphere. Laboratory blanks should be prepared and analyzed weekly to ensure quality of work.

(4) Several refined Jaffe wick washers for dissolving membrane filters.

(5) Miscellaneous EM supplies and chemicals, including carbon-coated 200-mesh copper grids, grid boxes, and chloroform.

(6) Sample collection equipment, including 37-mm-diameter or 47-mm-diameter filter holders, 0.4-μm (pore size) polycarbonate filters, 5.0-μm (pore size) cellulose ester membrane filters for back-up, a sampling pump with ancillary equipment, a tripod, critical orifices or flow meters, and a rain/wind shield.

DESCRIPTION OF METHODOLOGY

1. Type of Samples—Source

This protocol was originally developed for the EPA for measuring airborne asbestos (Samudra et al., 1977; Samudra et al., 1978). A broad interpretation of airborne has been to apply the term to samples obtained from ambient air (the original purpose), aerosolized source materials (such as the asbestos workplace environment, and fugitive dust emissions), bulk-air material (such as total suspended particulate (TSP) samples, dust, and powders) and any other type of sample obtained by nonrestrictive use of (1) collection of a volume of air, (2) separation from the air, and (3) concentration of the particulates onto a substrate. The airborne protocol has also been applied to samples collected in the regulatory areas of the EPA, as compared with, for example, the workplace environment (National Institute of Occupational Safety and Health), mining activities (U.S. Bureau of Mines), and shipboard atmosphere (Federal Maritime Administration).

The present methodology has been optimized for application specifically to samples collected from a volume of air in which the asbestos concentration is considered a minor component of the total particulate loading (other analytical methods are available for samples known to contain high concentrations of asbestos); and in which the particles are less than 15 μm in diameter, since particles greater than 15 μm either are not inhaled or are deposited in the upper respiratory tract and expelled, and preferably less than 10 μm in diameter as recommended by the Clean Air Scientific Advisory Committee (Hileman, 1981), since particles up to 10 μm can be absorbed by the alveolar

region of the lung. These concentration and size restrictions will preclude many air samples collected in an asbestos-processing environment and in bulk-air material from the complete methodology. However, such samples can still be examined with the TEM, within the limitations of the instrument, through changes in preparation techniques--provided the effects on the final results, such as fractionation of size and representativeness of the sample, are carefully considered.

2. Sample Collection and Transport

Sample Collection--

Sampling procedures vary depending on the nature of the sample, purpose of collection, analytical method to be used, sample substrate, and time and cost of sample collection relative to the total analytical effort. Nevertheless, the primary objective of sample collection always is to obtain a representative, unbiased sample.

Impingers, impaction devices, electrostatic precipitators, and thermal precipitators have been used in sample collection, but each has limitations. Presently, the preferred substrates are membrane filters, which are manufactured from different polymeric materials, including polycarbonate, mixed esters of cellulose, polystyrene, cellulose acetate, and cellulose nitrate. Polycarbonate membrane filters differ from the others in being thin, strong, and smooth-surfaced, and in having sieve-like construction (circular pores from top surface to the bottom). The other membrane filters are thicker, have irregular-surfaces, and have depth-filter construction (tortuous paths from top surface to bottom).

Consequently, polycarbonate filters have been selected for airborne asbestos analysis. The collection of small-sized particles (prefer less than 10 μm in diameter), the light loading of particulates, the uniform distribution of particulates attainable using a depth-type backing filter, the smooth surface and circular holes (which aid in determining size and instrument tilt axis), and the relative ease in grid transfer (thin and strong) minimize disadvantages of lack of retention and/or movement of large particles during handling. Other membrane materials, such as the cellulose ester type, are recommended for phase contrast and PLM, heavy particle loadings, and physical retention of large particles.

In microscopical analysis, uniformity of particulate distribution and loading is critical to success. Air samples are taken on 37-mm-diameter or 47-mm-diameter, 0.4-μm (pore size) polycarbonate membrane filters using the shiny, smooth side as the particle-capture surface. Cellulose ester-type membrane filters (pore size, 5.0 μm) are used to support the polycarbonate filter on the support pad (37-mm-diameter personal sampler) or on the support plate (47-mm-diameter holder).

Air monitoring cassettes (37-mm-diameter) of three-piece construction are available from several manufacturers. As with the 47-mm-diameter filters, loading the cassettes with the support pad, back-up filter, and 0.4 μm (pore size) polycarbonate filter should be carefully performed on a class-100 clean bench. Since the filters are held in place by pressure fit rather than by

screw tightening, air must not enter from the sides of the unit; a plastic band or tape (which can double as a label) should be used as a final seal.

Collecting airborne samples with proper loading requires experience. Each of the following techniques is useful in collecting airborne samples for direct microscopy, preserving representative sizes, without diluting particulate deposits:

(1) For long-term sampling at a site, test samples should be returned to the laboratory by express mail service, or air express service or by being hand-carried, and should then be analyzed by scanning electron microscopy.

(2) The estimated particulate loading (deposit is barely visible to the naked eye) should be bracketed by varying the filtration rate and using the same time, or by varying the time and using the same filtration rate.

(3) An automatic particle counter, such as a light-scattering instrument (0.3-μm detection) or a real-time mass monitor (0.1-μm detection), should be used to obtain an approximate particulate-loading level of the area.

Although any one of the three techniques will work, the suggested technique is to take the samples as a set, varying the sampling rates and using the same time so as to obtain filter samples with different particulate loadings. Each set is composed of a minimum of four 37-mm-diameter or 47-mm-diameter filter units--three for different particulate loadings (low, medium, high), and the fourth for a field blank. Suggested sampling rates are 0 for the field blank, 2.48 L/min for the low loading, 7.45 L/min for the medium, and 17.62 L/min for the high, for a 30 min sampling period using a 47-mm-diameter filter holder. Simultaneous sampling will provide at least one sample with a particulate loading suitable for direct EM analysis.

TSP's range from 10 $\mu g/m^3$ in remote, nonurban areas, to 60 $\mu g/m^3$ in near-urban areas, to 220 $\mu g/m^3$ in urban areas. However, for heavily polluted areas, TSP levels may reach 2000 $\mu g/m^3$. A loading of 5 to 10 μg per cm^2 of filter is adequate for EM analysis; values beyond 20 to 25 μg per cm^2 require a dilution treatment. As an example, for 47-mm-diameter filters at face velocities of 3.0 cm/s (2.48 L/min), 9.0 cm/s (7.45 L/min), and 21.2 cm/s (17.62 L/min), respectively, air volumes of 74.4 L, 223.5 L, and 528.6 L are sampled in 30 min. For a TSP level of 200 $\mu g/m^3$, 14.88 μg (1.07 $\mu g/cm^2$), 44.7 μg (3.23 $\mu g/cm^2$), and 105.7 μg (7.63 $\mu g/cm^2$), respectively, would be collected on 47-mm-diameter filters (which would have effective filtration areas of 13.85 cm^2). The sampling time could be increased to 60 min for areas having lower TSP levels, or reduced in a heavily polluted area (source emissions).

Airborne samples from emission sources contain coarse particles (above the respirable size) of large matrix structures, binder materials, road dust, clay minerals, fillers, and other materials. For these samples, a fifth filter unit can be added that has a size-selective inlet (cyclone, impactor, or elutriator) attached prior to the filter unit. The flow pattern and flow

rates of the tandem sampling arrangement must be checked before use. A satisfactory, tested combination presently used in California is a cyclone-filter unit with a D_{50} cut-off of 2.5 µm at 21.7 L/min, and a D_{50} cut-off of 3.5 µm at 15.4 L/min (John and Reischl, 1980). Additional sampling devices, such as impingers (used in biological sampling), impactors, and other designated filter units (for TSP, XRD, or x-ray fluorescence (XRF), for example) can be added to the system to obtain supplementary as well as interrelated data.

This expandable multifilter sampling unit, designated Hydra, offers the following advantages:

(1) It is small, inexpensive, and compact, so that an adult can easily handle it.

(2) It is efficiently designed, and includes a tripod, sampling pump, manifold, critical orifices, and a row of preloaded 37-mm-diameter or 47-mm-diameter filter holders. A rain/wind shield, size-selective cyclone-filter units, tubing, and other extras can be added as needed.

(3) Its sample preparation steps and handling are minimized.

(4) It allows complementary as well as supplementary analysis (TSP, size fractionation, bacteria, and XRF, for example), although additional air sampling capacity is required.

(5) It accommodates ambient air and source emission samples, with or without a size-selective inlet.

(6) It allows synchronous sampling in several places in the vicinity following the same sampling procedure, thereby accommodating particulate concentration fluctuations.

(7) It includes filter holders that serve as transport and storage units.

Hydra's disadvantages are a short sampling period, which may record an episode; a small sampling quantity or volume, which may not indicate the presence of asbestos fibers; and a detection limit of 2×10^4 fibers/m^3 for sampling 1 m^3 of air with the 47-mm-diameter filter.

Using 8 inch x 10 inch, or 102-mm-diameter filter sizes, is not recommended. The sampling units are designed for purposes other than microscopy. Interchanging the type of sample substrate filter (glass fiber or paper to polycarbonate) does not correct the inherent problems of filter size and sampling unit.

Sample Storage and Transport—

Once the sample is acquired, its integrity must be assured, and contamination and loss of fibers prevented, until it is examined under the EM. The low cost and small size of the 37-mm-diameter and 47-mm-diameter filter holders enables them to be used as combination storage and transport containers. The filter holders should be maintained in a horizontal position

during storage and transport to the laboratory so that the particulate-loaded filters can be removed under optimally controlled conditions in the laboratory.

For 47-mm-diameter holders (open-face) to be used in transport or storage, the screw cap is carefully removed, and the shiny, waxy, stiff separator paper used to keep the polycarbonate filters apart is carefully placed on the retaining ring. The cap is then carefully screwed back on so that the separator paper seals and protects the particulate-loaded filter without touching it. The 37-mm-diameter, three-piece filter holder (aerosol monitor) is used in its open-face position, and capped after usage for transport and storage.

When the more expensive 47-mm-diameter holder is to be re-used immediately, the particulate-loaded filter should be carefully removed and placed in a 47-mm-diameter Petri-slide (such as that manufactured by the Millipore Corp.*) This transfer takes place in the field rather than in the laboratory, so that the Petri-slide should be taken into the field. The 37-mm-diameter filter holder or the 47-mm-diameter holder/Petri-slide should be secured and all necessary sample identification marks and symbols applied to the holder.

3. Sample Preparation for Analysis--Grid Transfer

Carbon-Coating the Filter--

The polycarbonate filter, with the sample deposit and suitable blanks, should be coated with carbon as soon as possible after sampling is completed. To begin this procedure, the particulate-loaded 47-mm-diameter polycarbonate filter is removed from the holder and transferred carefully to an open-faced 47-mm-diameter Petri-slide for carbon-coating in the vacuum evaporator (see Figure A1, Appendix A). If the 47-mm-diameter filter is already in the Petri-slide, the cover is replaced with an open-face cover, minimizing filter disruption. The 37-mm-diameter filter is left in the holder, but the upper lid is removed to create an open-faced filter. The open-faced holders are placed on the rotating turntable in the vacuum evaporator for carbon-coating. Figure A2 shows the multiple-coating arrangement in the evaporator; Figure A3 shows a close-up of the 37-mm-diameter and the modified 47-mm-diameter holders for carbon-coating.

For archival filters and those of larger sizes, portions of about 2.5 cm x 2.5 cm should be cut midway between the center and edge using a scalpel. The portions are then attached with cellophane tape to a clean glass microscope slide and placed on the turntable in the vacuum evaporator for coating.

Any high-vacuum carbon evaporator may be used to carbon-coat the filters (CAUTION: carbon sputtering devices should not be used). Typically, the electrodes are adjusted to a height of 10 cm above the level of the filters. A spectrographically pure carbon electrode sharpened to a neck of 0.1 cm x 0.5 cm is used as the evaporating electrode. The sharpened electrode is

* Millipore Corp., 80-T Ashby Rd., Bedford, Mass. 01730

placed in its spring-loaded holder so that the neck rests against the flat surface of a second carbon electrode.

The manufacturer's instructions should be followed to obtain a vacuum of about 1.33×10^{-3} Pa (1×10^{-5} torr) in the bell jar of the evaporator. With the turntable in motion, the neck of the carbon electrode is evaporated by increasing the electrode current to about 15 A in 10 s, followed by 20 to 25 A for 25 to 30 s. If the turntable is not used during carbon evaporation, the particulate matter may not be coated from all sides, resulting in an undesirable shadowing effect. The evaporation should proceed in a series of short bursts until the neck of the electrode is consumed. Continuous prolonged evaporation should be avoided, since overheating and consequent degradation of the polycarbonate filter may occur, impeding the subsequent step of dissolving the filter. The evaporation process may be observed by viewing the arc through welders goggles (CAUTION: never look at the arc without appropriate eye protection). Preliminary calculations show that a carbon neck of 5 mm^3 volume, when evaporated over a spherical surface 10 cm in radius, will yield a carbon layer that is 40 nm thick.

Following carbon-coating, the vacuum chamber is slowly returned to ambient pressure, and the filters are removed and placed in their respective holders or in clean, marked Petri dishes for storage on a clean bench.

Transfer of the Sample to the EM Grid—

Transferring the collected particulates from the carbon-coated polycarbonate filter to an EM grid is accomplished in a clean room or on a class-100 clean bench. The transfer is made in a Jaffe wick washer, which is usually a glass Petri dish containing a substrate to support the EM grid/carbon-coated membrane filter combination. Solvent is added to a level to just wet the combination and cause gentle dissolution of the membrane with minimum loss or dislocation of the particulates, resulting in a membrane-free EM grid with particles embedded in the carbon film coating. The substrate support can be stainless steel mesh bridges, filter papers, urethane foams, or combinations of these.

The refined Jaffe wick washer is described as follows:

(1) The glass Petri dish (diameter, 10 cm; height, 1.5 cm) is made airtight by grinding the top edge of the bottom dish with the bottom of the cover dish, with water and Carborundum* powder (80 mesh); this creates a ground-glass seal (closer fit) and minimizes the need to refill the Petri dish with added solvent. (The usual glass Petri dish was found not to retain the solvent for long periods of time, and unless the wicking substrate is kept continuously wet, poor solubility of the membrane filter results, leading to a poor-quality EM grid).

* Carborundum is a registered trademark of the Carborundum Co., Carborundum Center, Niagara Falls, N.Y. 14302.

(2) A combination of foam and a single sheet of 9-cm filter paper is used as the substrate support. A 3-cm x 3-cm x 0.6-cm piece of polyurethane foam (the packing in Polaroid film boxes) is cut and placed in the bottom dish. A 0.5-inch V-shaped notch is cut into the filter paper; the notch is oriented in line with the side of the foam, creating a well for adding solvent. Spectrographic-grade chloroform (solvent) is poured into the Petri dish through the notch until it is level with the top of the foam (also level with the paper). The foam will swell, and care is needed to avoid adding solvent above the filter paper.

(3) On top of the filter paper, pieces of 100-mesh stainless steel screen (0.6 cm x 0.6 cm) are placed, usually in two rows, to make several grid transfers at one time (for such uses as replicas), and to facilitate maintenance of proper identity of each transfer.

(4) A 3-mm section (usually midway between the center and edge) of the carbon-coated polycarbonate filter is cut in a rocking motion with a scalpel. The section may be a square, rectangle, or triangle, and should just cover the 3-mm EM grid.

(5) A section is laid carbon-side down on a 200-mesh carbon-coated EM grid. (Alternatively, Formvar-coated* grids or uncoated EM grids may be used. Here, the carbon coating on the polycarbonate filter forms the grid substrate.) Minor overlap or underlap of the grid by the filter section can be tolerated, since only the central 2-mm portion of the grid is scanned in the microscope. The EM grid and filter combination is picked up at the edges with the tweezers and carefully laid on the damp 100-mesh stainless steel screen. The EM grid-filter combination will immediately "wet out" and remain on the screen.

(6) Once all specimens are placed in the washer, more solvent is carefully added through the notch to maintain the liquid level so that it just touches the top of the paper filter. Raising the solvent level any higher may float the EM grid off the mesh or displace the polycarbonate filter section.

(7) The cover is placed in the washer and oriented in place over the specimen, and a map of the filter/grid/screen arrangement is made on the glass cover and in the logbook.

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* Formvar is a registered trademark of the Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, Mo.

(8) Solvent (chloroform) is added periodically to maintain the level within the washer until the filter is completely dissolved by the wicking action (24 to 48 h).

(9) The temperature in the room must remain relatively constant to minimize condensation of solvent on the bottom of the cover and subsequent falling of solvent drops on the EM grid. Should day-night or other temperature differentials occur, solvent condensation on the under-surface of the cover can be minimized by placing the Jaffe washer at a slight tilt (three glass slides under one edge of the Petri dish parallel to the row of grids) to allow the condensation drops to flow toward the lower edge rather than fall on the EM grids. At temperatures lower than 20°C (68°F), the complete filter solution may take longer than 72 h.

(10) After the polymer is completely dissolved, the stainless steel mesh screen with the EM grid is picked up while wet and set on lens paper tacked to the bottom of a separate Petri dish. The EM grid is then lifted from and placed next to the screen to dry. When all traces of solvent have evaporated, the grid is stored in a grid box and identified by location and grid box in the logbook.

Figure A4 illustrates the Jaffe wick washer method; Figure A5 shows the washer. The foam/filter combination is currently preferred, as is use of a closely fitted (by means of the ground-glass seal) Petri dish.

Gold Coating--

An additional step will aid in subjectively evaluating the SAED pattern. This step is required for specimens from the upper Great Lakes area and for those of unknown origins. After the particulates on the filter are transferred to the EM grid, the grid is held to a glass slide with double stick tape for gold-coating in the vacuum evaporator. Several EM grids may be taped to the glass slide for coating at one time. Approximately 10 mm of 0.015-cm-diameter (0.006-inch) pure gold wire is placed in a tungsten basket (10 cm from the rotating table holding the EM grids) and evaporated onto the grid.

The thin gold-coating establishes an internal standard for SAED analysis. For some mineral species, an internal standard will clarify visual identification of the pattern of a fibrous particulate as being or not being an amphibole species (for example, minnesotaite as opposed to amosite). With experience, differentiation in SAED patterns can be observed. For samples of known geographic origins, gold-coating is optional, since the additional coating hinders observation and identification of small-diameter chrysotile fibers.

4. TEM Examination and Data Collection

Low Magnification Examination of Grids--

Figure A6 shows a modern TEM. The grid is observed in the TEM at magnifications of 250X and 1000X to determine its suitability for detailed

study at higher magnification. The grid is rejected and a new grid used if: (1) the carbon film over a majority of the grid openings is damaged and not intact; (2) the specimen is dark due to incomplete dissolution of the polycarbonate filter; or (3) the particulate loading is too light (unless a blank) or too heavy with particle-particle interactions or overlaps.

TEM Analysis (Morphology and SAED)--

The following guidelines are observed for consistency in the analytical protocol:

(1) Magnification at the fluorescent screen is determined by calibration with a diffraction-grating replica in the specimen holder.

(2) A field of view or "gate" is defined. On some microscopes, the central rectangular portion of the fluorescent screen, which is lifted for photographic purposes, is convenient to use. On others, a scribed circle or the entire circular screen may be used as the field of view. The area of the field of view must be accurately measurable.

(3) The grid opening is selected on a random basis.

(4) The analysis, morphology, and SAED are performed at a tilt angle of 0°.

(5) The recommended instrument settings are: accelerating voltage, 100 kV; beam current, 100 μA; film magnification, 20,000X (which is equivalent to 16,000X on the fluorescent screen for this instrument); and concentric circles of radii 1, 2, 3, and 4 cm on the fluorescent screen.

(6) The grid opening is measured at 1000X.

(7) Since asbestos fibers are found isolated as well as with each other or with other particles in varying arrangements, the fibrous particulates are characterized as asbestos structures:

Fiber (F) is a particle with an aspect ratio of 3:1 or greater, with substantially parallel sides.

Bundle (B) is a particulate composed of fibers in a parallel arrangement, with each fiber closer than the diameter of one fiber.

Cluster (Cl) is a particulate with fibers in a random arrangement such that all fibers are intermixed and no single fiber is isolated from the group.

Matrix (M) is a fiber or fibers with one end free and the other end embedded or hidden by a particulate.

Combinations of structures, such as matrix and cluster, matrix and bundle, or bundle and cluster, are categorized by the dominant fiber quality--cluster, bundle, or matrix.

(8) Counting rules for single fibers are:

(a) Particulates meeting the definition of fiber are isolated by themselves. With this definition, edge view of flakes, fragments from cleavage planes, and scrolls, for example, may be counted as fibers.

(b) Count as single entities if separation is equal to or greater than the diameter of a single fiber.

(c Count as single entities if three ends can be seen.

(d) Count as single entities if four ends can be seen.

(e) In general, fibers that touch or cross are counted separately.

(f) Two or more fibers are counted as a bundle if the distances between fibers are less than the diameter of a single fiber, or if the ends cannot be resolved.

(g) Fibrils attached longitudinally to a fiber are counted as part of the fiber and the size (width) is estimated based on the fiber-to-fibril relationship.

(h) A fiber partially hidden by grid wires (one or two ends) is counted, but labeled as an X-fiber. If the number of X-fibers is more than 20% of the fibers identified as asbestos, a larger-mesh EM grid should be used, such as 100 mesh (about 200 μm wide).

(9) Sizing rules for asbestos structures are:

(a) For fibers, widths and lengths are obtained by orienting the fibers to the inscribed circles on the fluorescent screen. Since estimates are within ±1 mm, small-diameter fibers have greater margins of error. Fibers less than 1 mm at the fluorescent screen magnification level are characterized as being 1 mm. A cylindrical shape is assumed for fibers. X-fibers are sized by measuring their entire visible portions in the grid opening.

(b) Bundles and clusters are sized by estimating their widths and lengths. The sum of individual diameters is used to obtain the total width, and an average length for the total length. A laminar-sheet shape

is assumed, with the average diameter of the individual fiber as the thickness.

(c) Matrices are sized by adding the best estimates of individual fiber components. A laminar or sheet structure is assumed for volume calculation.

(10) The method of sizing is as follows:

(a) An asbestos structure is recognized, and its location in the rectangular "gate" relative to the sides, inscribed circles, and other particulates, is memorized.

(b) The structure is moved to the center for SAED observation and sizing.

(c) Sizing is performed using the inscribed circles. If the structure, such as a fiber, extends beyond the rectangular "gate" (field of view), it is superimposed across the series of concentric circles (several times, if necessary) until the entire structure is measured.

(d) The structure is returned to its original location by recall of the location, and scanning is continued.

Figure A7 illustrates some of the counting and morphology guidelines used in determining asbestos structures.

TEM Procedure--

The TEM procedure is as follows:

(1) EM grid quality is assessed at 250X.

(2) Particulate loading is assessed at 1000X.

(3) A grid opening is selected at random, examined at 1000X, and sized.

(4) A series of parallel traverses is made across the grid opening at the film magnification of 20,000X. Starting at one corner, and using the tilting section of the fluorescent screen as a "gate" or "chute," the grid opening is traversed. Movement through the "gate" is not continuous, but rather is a stop/go motion. On reaching the end of one traverse, the image is moved the width of one "gate," and the traverse is reversed. These parallel traverses are made until the entire grid opening has been scanned.

(5) Asbestos structures are identified morphologically and counted as they enter the "gate."

(6) The asbestos structure is categorized as fiber (with or without X-) bundle, cluster, or matrix, and sized through use of the inscribed circles.

(7) The structure (individual fiber portion) is centered and focused, and the SAED pattern is obtained through use of the field-limiting aperture.

(a) SAED patterns from single fibers of asbestos minerals fall into distinct groups. The chrysotile asbestos pattern has characteristic streaks on layer lines other than the central line, and some streaking also on the central line. Spots of normal sharpness are present on the central layer line and on alternate lines (that is, 2nd, 4th etc.) The repeat distance between layer lines is about 0.53 nm.

(b) Amphibole asbestos fiber patterns show layer lines formed by very closely spaced dots, and have repeat distances between layer lines also of about 0.53 nm. Streaking in layer lines is occasionally present due to crystal structure defects.

(c) Transmission electron micrographs and SAED patterns obtained with asbestos standard samples should be used as guides to fiber identification. An example is the "Asbestos Fiber Atlas" (Mueller et al., 1975).

(8) From visual examination of the SAED pattern, the structure is classified as belonging to one of four categories: (1) chrysotile, (2) amphibole group (includes amosite, crocidolite, anthophyllite, tremolite, and actinolite), (3) ambiguous (incomplete spot patterns), or (4) no identification. SAED patterns cannot be inspected for some fibers. Reasons for the absence of a recognizable diffraction pattern include contamination of the fiber, interference from nearby particles, fibers that are too small or too thick, and nonsuitable orientation of the fiber. Some chrysotile fibers are destroyed in the electron beam, resulting in patterns that fade away within seconds of being formed. Some patterns are very faint and can be seen only under the binocular microscope. In general, the shortest available camera length must be used, and the objective lens current may need to be adjusted to give optimum pattern visibility for correct identification. A 20-cm camera length and a 10X binocular are recommended for inspecting the SAED pattern on the tilted screen.

(9) Additional grid openings are selected, scanned, and counted until either the total number of structures counted exceeds 100 per known area or a minimum of 10 grid openings has been scanned, whichever is first.

(10) The TEM data should be recorded in a systematic form so that it can be processed rapidly. Sample information, instrument parameters, and the sequence of operations should be tabulated for ease in data reduction and subsequent reporting of results. Figure A8 shows an example of a data sheet used in Level I analysis.

Figure A9 illustrates the method of scanning a full-grid opening. The "field of view" method of counting previously included in the provisional methodology, which is based on randomly selected fields of view, has been discontinued. Originally, the method was recommended for medium loading level on the filter (50 to 300 fibers per grid opening). However, if samples are collected at three different loading levels and the optimum is selected, this medium loading on the filter will not be used. Samples with grid openings containing 50 to 300 fibers may be used as laboratory fiber preparations or selected source samples, but in field samples the particulate loading is usually of much higher concentration than the fiber. Filter loading is characterized by the particulate concentration, not by fiber concentration.

5. Data Reduction and Reporting of Results

Data Reduction—

From the data sheet, size measurements are converted to microns (16,000X screen magnification), mass of asbestos structure is calculated, and other characterizing parameters are calculated through use of a hand calculator or computer. (Appendix B, an example of a computer printout from Level I analysis, shows reduced data--that is, what was found on the specified number of grid openings or area examined.) These measurements are summarized and related to the volume of air sampled and the total effective filtration area (area of deposit). Size measurements of X-fibers may be doubled and noted, or kept as a separate category.

Fiber number concentration is calculated from the equation

$$\text{Fibers/m}^3 = \frac{\text{Total no. of fibers}}{\text{No. of EM fields}} \times \frac{\text{Total effective filter area, cm}^2}{\text{Area of an EM field, cm}^2} \times \frac{1}{\text{Volume of air sampled, m}^3}$$

The number of X-fibers, bundles, clusters, and matrices are calculated in a similar manner. X-fibers may be included with fibers if they are few in

number. Similarly, their corresponding mass (from their size measurements) may be included.

Fiber mass for each type of asbestos (chrysotile or amphibole) in the sample is calculated by assuming that both chrysotiles and amphiboles have circular cross-sections (cylindrical shape) and that the width measurements are one diameter. The density of chrysotile is assumed to be 2.6 g/cm^3, and of amphiboles to be 3.0 g/cm^3. The individual mass is calculated from the equation

$$\text{Mass, } \mu g = \frac{\pi}{4} \times (\text{length, } \mu m) \times (\text{diameter, } \mu m)^2 \times (\text{density, } g/cm^3) \times 10^{-6}$$

The total mass concentration of fibers for each type of asbestos is then calculated from the total mass of all the individual fibers of that type.

The individual masses of bundles, clusters, and matrices are calculated by assuming a laminar or sheet-like structure with an average thickness of the fiber make-up of the structure. Again, the density of chrysotile is assumed to be 2.6 g/cm^3, and of amphiboles to be 3.0 g/cm^3. The individual masses are calculated from the equation

$$\text{Mass, } \mu g = (\text{length, } \mu m) \times (\text{width, } \mu m) \times (\text{thickness, } \mu m) \times (\text{density, } g/cm^3) \times 10^{-6}$$

The total mass for each type of structure for each type of asbestos is the sum of all the individual masses.

Other characterizing parameters of the asbestos structures are: (1) length and width distribution of fibers, (2) aspect ratio distribution of fibers, and (3) relationships of fibers, bundles, clusters, and matrices.

Reporting of Results—

The data in their acquired and reduced forms are reported as summarized, or, depending on the purpose of the analysis, are further reduced to present the interrelationships of the various characterizing parameters. Again, the Level I methodology is a monitoring or screening technique, and its limitations, such as the possibility of "false positives" and misidentification, should be noted.

6. Quality Control/Quality Assurance

Sampling procedures will vary depending on the type of sample, objectives of the sampling, and time/cost factors. The primary goals of sampling are to obtain a representative sample at the location and time of sampling, and to maintain sample integrity. The sampling team will have written sampling

procedures, and the field chief and/or designated individual will be responsible for all record-keeping (including sample identification, labeling, logging of data, site description, and meteorological conditions), pre- and post-collection checks, and continuous sample custody and sign-outs until the sample is delivered to the laboratory and transferred to the appropriate quality assurance officer (QAO). Verification of sampling times, flow rates, equipment calibration, and taking of field blanks will be checked and recorded in the field logbook.

Samples are turned over to the QAO for logging into a project logbook. Each sample is carefully examined for gross features, such as tears, breaks, and overall condition of container. The QAO registers the as-received sample number and other designated information, and assigns a simple internal code number that will accompany the sample through the preparation stage, grid transfer, grid analysis, data reduction, and reporting of results.

After being logged into the project logbook, the sample is transferred to the custody of the electron microscopy staff, where every precaution is taken to maintain sample integrity and to prevent contamination and loss of collected particulates. During storage and transport, the filters in their respective holders are maintained in a horizontal position at all times.

The sample logging, handling, and storing procedures ensure that all samples can be readily located and identified throughout the course of a program. The QAO has divisional responsibility for QC/QA activities, and must see that the laboratory maintains high standards. He must be aware of current standards of analysis, and must ensure that internal quality control standards, instrument calibration, and records of samples and completed analyses are kept for ease of later retrieval and use.

For quality control, internal laboratory blanks are analyzed at least once a week, which may or may not coincide with a sample batch blank. In addition, a magnification calibration of the EM using a carbon grating replica (2,160 lines per mm) is performed once a week. The results are recorded in an EM instrument log, along with other routine instrumental performance checks. All photographs, TEM, SEM, and STEM images are recorded in a photo log. These QC results are documented for inspection by the QAO.

SECTION 5

LEVEL II ANALYSIS

SUMMARY OF PROTOCOL

Level II analysis is a regulatory technique consisting of Level I analysis plus chemical elemental analysis. Morphology, size, SAED pattern, and chemical analysis are obtained sequentially. By a process of elimination, mineral fibers are identified as chrysotile, amphibole, ambiguous, or "no-identity" by morphology and SAED pattern. X-ray elemental analysis is used to categorize the amphibole fibers, identify the ambiguous fibers, and confirm or validate chrysotile fibers.

Level II analysis is summarized as follows:

(1) A known volume of air is passed through a polycarbonate membrane filter (pore diameter, 0.4 μm; filter diameter, 37 or 47 mm) to obtain approximately 5 to 10 μg of particulates per cm^2 of filter surface.

(2) The particulate-laden filter is transported in its own filter holder.

(3) The filter is carbon-coated in the holder.

(4) The particulates are transferred to an EM grid using a refined Jaffe wick washer.

(5) The EM grid, containing the particulates, is gold-coated lightly.

(6) The EM grid is examined under low magnification (250X to 1000X) followed by high-magnification (16,000X on the fluorescent screen) search and analysis.

(7) A known area (measured grid opening) is scanned, and the fibrous structures (fibers, bundles, clusters, and matrices) are counted, sized, and identified as to asbestos type (chrysotile, amphibole, ambiguous, or no identity) by morphology and by observing the SAED pattern; and finally by elemental analysis using EDS.

(8) The observations are recorded--a minimum of 100 fibrous structures or 10 grid openings, whichever is first.

(9) The data are reduced and the results reported.

EQUIPMENT, FACILITIES, AND SUPPLIES

The following items are required for Level I analysis:

(1) A modern 100-kV TEM equipped with an EDS. A scanning accessory as found in a STEM will increase the versatility and analytical capability for very small fibers and for fibers adjacent to other particulate matter. The microscope should be equipped with the fluorescent viewing screen inscribed with graduation of known radii to estimate the length and width of fibrous particulates.

(2) A vacuum evaporator with a turntable for rotating specimens during coating, for such uses as carbon-coating polycarbonate filters, gold-coating EM grids, and preparing carbon-coated EM grids.

(3) An EM preparation room adjacent to the room housing the EM. This room should either be a clean-room facility, or contain a laminar-flow class-100 clean bench to minimize contamination duing EM grid preparation. Filter handling and transfer to EM grids should be performed in a clean atmosphere. Laboratory blanks should be prepared and analyzed weekly to ensure quality of work.

(4) Several refined Jaffe wick washers for dissolving membrane filters.

(5) Miscellaneous EM supplies and chemicals, including carbon-coated 200-mesh copper grids, grid boxes, and chloroform.

(6) Sample collection equipment, including 37-mm-diameter or 47-mm-diameter filter holders, 0.4-μm (pore size) polycarbonate filters, 5.0-μm (pore size) cellulose ester membrane filters for back-up, a sampling pump with ancillary equipment, a tripod, critical orifices or flow meters, and a rain/wind shield.

DESCRIPTION OF METHODOLOGY

1. Type of Samples—Source

This protocol is an expansion of the method originally developed for the EPA for measuring airborne asbestos (Samudra et al., 1977; Samudra et al., 1978). A broad interpretation of airborne has been to apply the term to samples obtained from ambient air (the original purpose), aerosolized source materials (such as the asbestos workplace environment, and fugitive dust emissions), bulk-air material (such as total suspended particulate (TSP) samples, dust, and powders) and any other type of sample obtained by nonrestrictive use of (1) collection of a volume of air, (2) separation from the air, and (3) concentration of the particulates onto a substrate. The airborne protocol has also been applied to samples collected in the regulatory areas of the EPA, as compared with, for example, the workplace environment (National

Institute of Occupational Safety and Health), mining activities (U.S. Bureau of Mines), and shipboard atmosphere (Federal Maritime Administration).

The present methodology has been optimized for application specifically to samples collected from a volume of air in which the asbestos concentration is considered a minor component of the total particulate loading (other analytical methods are available for samples known to contain high concentrations of asbestos); and in which the particles are less than 15 μm in diameter, since particles greater than 15 μm either are not inhaled or are deposited in the upper respiratory tract and expelled, and preferably less than 10 μm in diameter as recommended by the Clean Air Scientific Advisory Committee (Hileman, 1981), since particles up to 10 μm can be absorbed by the alveolar region of the lung. These concentration and size restrictions will preclude many air samples collected in an asbestos-processing environment and in bulk-air material from the complete methodology. However, such samples can still be examined with the TEM, within the limitations of the instrument by changes in preparation techniques--provided the effects on the final results, such as fractionation of size and representativeness of the sample, are carefully considered.

2. Sample Collection and Transport

Sample Collection—

Sampling procedures vary depending on the nature of the sample, purpose of collection, analytical method to be used, sample substrate, and time and cost of sample collection relative to the total analytical effort. Nevertheless, the primary objective of sample collection always is to obtain a representative, unbiased sample.

Impingers, impaction devices, electrostatic precipitators, and thermal precipitators have been used in sample collection, but each has limitations. Presently, the preferred substrates are membrane filters, which are manufactured from different polymeric materials, including polycarbonate, mixed esters of cellulose, polystyrene, cellulose acetate, and cellulose nitrate. Polycarbonate membrane filters differ from the others in being thin, strong, and smooth-surfaced, and in having sieve-like construction (circular pores from top surface to the bottom). The other membrane filters are thicker, have irregular-surfaces, and have depth-filter construction (tortuous paths from top surface to bottom).

Consequently, polycarbonate filters have been selected for airborne asbestos analysis. The collection of small-sized particles (prefer less than 10 μm in diameter), the light loading of particulates, the uniform distribution of particulates attainable using a depth-type backing filter, the smooth surface and circular holes (which aid in determining size and instrument tilt axis), and the relative ease in grid transfer (thin and strong) minimize disadvantages of lack of retention and/or movement of large particles during handling. Other membrane materials, such as the cellulose ester type, are recommended for phase contrast and PLM, heavy particle loadings, and physical retention of large particles.

In microscopical analysis, uniformity of particulate distribution and loading is critical to success. Air samples are taken on 37-mm-diameter or 47-mm-diameter, 0.4-μm (pore size) polycarbonate membrane filters using the shiny, smooth side as the particle-capture surface. Cellulose ester-type membrane filters (pore size, 5.0 μm) are used to support the polycarbonate filter on the support pad (37-mm-diameter personal sampler) or on the support plate (47-mm-diameter holder).

Air monitoring cassettes (37-mm-diameter) of three-piece construction are available from several manufacturers. As with the 47-mm-diameter filters, loading the cassettes with the support pad, back-up filter, and 0.4 μm (pore size) polycarbonate filter should be carefully performed on a class-100 clean bench. Since the filters are held in place by pressure fit rather than by screw tightening, air must not enter from the sides of the unit; a plastic band or tape (which can double as a label) should be used as a final seal.

Collecting airborne samples with proper loading requires experience. Each of the following techniques is useful in collecting airborne samples for direct microscopy, preserving representative sizes, without diluting particulate deposits:

(1) For long-term sampling at a site, test samples should be returned to the laboratory by express mail service, or air express service or by being hand-carried, and should then be analyzed by scanning electron microscopy.

(2) The estimated particulate loading (deposit is barely visible to the naked eye) should be bracketed by varying the filtration rate and using the same time, or by varying the time and using the same filtration rate.

(3) An automatic particle counter, such as a light-scattering instrument (0.3-μm detection) or a real-time mass monitor (0.1-μm detection), should be used to obtain an approximate particulate-loading level of the area.

Although any one of the three techniques will work, the suggested technique is to take the samples as a set, varying the sampling rates and using the same time so as to obtain filter samples with different particulate loadings. Each set is composed of a minimum of four 37-mm-diameter or 47-mm-diameter filter units--three for different particulate loadings (low, medium, high), and the fourth for a field blank. Suggested sampling rates are 0 for the field blank, 2.48 L/min for the low loading, 7.45 L/min for the medium, and 17.62 L/min for the high, for a 30 min sampling period using a 47-mm-diameter filter holder. Simultaneous sampling will provide at least one sample with a particulate loading suitable for direct EM analysis.

TSP's range from 10 $\mu g/m^3$ in remote, nonurban areas, to 60 $\mu g/m^3$ in near-urban areas, to 220 $\mu g/m^3$ in urban areas. However, for heavily polluted areas, TSP levels may reach 2000 $\mu g/m^3$. A loading of 5 to 10 μg per cm^2 of filter is adequate for EM analysis; values beyond 20 to 25 μg per cm^2 require a dilution treatment. As an example, for 47-mm-diameter filters at face velocities of 3.0 cm/s (2.48 L/min), 9.0 cm/s (7.45 L/min), and 21.2 cm/s

(17.62 L/min), respectively, air volumes of 74.4 L, 223.5 L, and 528.6 L are sampled in 30 min. For a TSP level of 200 μg/m^3, 14.88 μg (1.07 μg/cm^2), 44.7 μg (3.23 μg/cm^2), and 105.7 μg (7.63 μg/cm^2), respectively, would be collected on 47-mm-diameter filters (which would have effective filtration areas of 13.85 cm^2). The sampling time could be increased to 60 min for areas having lower TSP levels, or reduced in a heavily polluted area (source emissions).

Airborne samples from emission sources contain coarse particles (above the respirable size) of large matrix structures, binder materials, road dust, clay minerals, fillers, and other materials. For these samples, a fifth filter unit can be added that has a size-selective inlet (cyclone, impactor, or elutriator) attached prior to the filter unit. The flow pattern and flow rates of the tandem sampling arrangement must be checked before use. A satisfactory, tested combination presently used in California is a cyclone-filter unit with a D_{50} cut-off of 2.5 μm at 21.7 L/min, and a D_{50} cut-off of 3.5 μm at 15.4 L/min (John and Reischl, 1980). Additional sampling devices, such as impingers (used in biological sampling), impactors, and other designated filter units (for TSP, XRD, or x-ray fluorescence (XRF), for example) can be added to the system to obtain supplementary as well as inter-related data.

This expandable multifilter sampling unit, designated Hydra, offers the following advantages:

(1) It is small, inexpensive, and compact, so that an adult can easily handle it.

(2) It is efficiently designed, and includes a tripod, sampling pump, manifold, critical orifices, and a row of preloaded 37-mm-diameter or 47-mm-diameter filter holders. A rain/wind shield, size-selective cyclone-filter units, tubing, and other extras can be added as needed.

(3) Its sample preparation steps and handling are minimized.

(4) It allows complementary as well as supplementary analysis (TSP, size fractionation, bacteria, and XRF, for example), although additional air sampling capacity is required.

(5) It accommodates ambient air and source emission samples, with or without a size-selective inlet.

(6) It allows synchronous sampling in several places in the vicinity following the same sampling procedure, thereby accommodating particulate concentration fluctuations.

(7) It includes filter holders that serve as transport and storage units.

Hydra's disadvantages are a short sampling period, which may catch an episode; a small sampling quantity or volume, which may not indicate the presence of asbestos fibers; and a detection limit of 2 x 10^4 fibers/m^3 for sampling 1 m^3 of air with the 47-mm-diameter filter.

Using 8 inch x 10 inch, or 102-mm-diameter filter sizes, is not recommended. The sampling units are designed for purposes other than microscopy. Interchanging the type of sample substrate filter (glass fiber or paper to polycarbonate) does not correct the inherent problems of filter size and sampling unit.

Sample Storage and Transport—

Once the sample is acquired, its integrity must be assured, and contamination and loss of fibers prevented, until it is examined under the EM. The low cost and small size of the 37-mm-diameter and 47-mm-diameter filter holders enables them to be used as combination storage and transport containers. The filter holders should be maintained in a horizontal position during storage and transport to the laboratory so that the particulate-loaded filters can be removed under optimally controlled conditions in the laboratory.

For 47-mm-diameter holders (open-face) to be used in transport or storage, the screw cap is carefully removed, and the shiny, waxy, stiff separator paper used to keep the polycarbonate filters apart is carefully placed on the retaining ring. The cap is then carefully screwed back on so that the separator paper seals and protects the particulate-loaded filter without touching it. The 37-mm-diameter, three-piece filter holder (aerosol monitor) is used in its open-face position, and capped after usage for transport and storage.

When the more expensive 47-mm-diameter holder is to be reused immediately, the particulate-loaded filter should be carefully removed and placed in a 47-mm-diameter Petri-slide (such as that manufactured by the Millipore Corp.*). This transfer takes place in the field rather than in the laboratory, so that the Petri-slide should be taken into the field. The 37-mm-diameter filter holder or the 47-mm-diameter holder/Petri-slide should be secured and all necessary sample identification marks and symbols applied to the holder.

3. Sample Preparation for Analysis—Grid Transfer

Carbon-Coating the Filter—

The polycarbonate filter, with the sample deposit and suitable blanks, should be coated with carbon as soon as possible after sampling is completed. To begin this procedure, the particulate-loaded 47-mm-diameter polycarbonate filter is removed from the holder and transferred carefully to an open-faced 47-mm-diameter Petri-slide for carbon-coating in the vacuum evaporator (see Figure A1, Appendix A). If the 47-mm-diameter filter is already in the Petri-slide, the cover is replaced with an open-face cover, minimizing filter disruption. The 37-mm-diameter filter is left in the holder, but the upper lid is removed to create an open-faced filter. The open-faced holders are placed on the rotating turntable in the vacuum evaporator for carbon-coating. Figure A2 shows the multiple-coating

* Millipore Corp., 80-T Ashby Rd., Bedford, Mass. 01730

arrangement in the evaporator; Figure A3 shows a close-up of the 37-mm-diameter and the modified 47-mm-diameter holders for carbon-coating.

For archival filters and those of larger sizes, portions of about 2.5 cm x 2.5 cm should be cut midway between the center and edge using a scalpel. The portions are then attached with cellophane tape to a clean glass microscope slide and placed on the turntable in the vacuum evaporator for coating.

Any high-vacuum carbon evaporator may be used to carbon-coat the filters (CAUTION: carbon sputtering devices should not be used). Typically, the electrodes are adjusted to a height of 10 cm above the level of the filters. A spectrographically pure carbon electrode sharpened to a neck of 0.1 cm x 0.5 cm is used as the evaporating electrode. The sharpened electrode is placed in its spring-loaded holder so that the neck rests against the flat surface of a second carbon electrode.

The manufacturer's instructions should be followed to obtain a vacuum of about 1.33×10^{-3} Pa (1×10^{-5} torr) in the bell jar of the evaporator. With the turntable in motion, the neck of the carbon electrode is evaporated by increasing the electrode current to about 15 A in 10 s, followed by 20 to 25 A for 25 to 30 s. If the turntable is not used during carbon evaporation, the particulate matter may not be coated from all sides, resulting in an undesirable shadowing effect. The evaporation should proceed in a series of short bursts until the neck of the electrode is consumed. Continuous prolonged evaporation should be avoided, since overheating and consequent degradation of the polycarbonate filter may occur, impeding the subsequent step of dissolving the filter. The evaporation process may be observed by viewing the arc through welders goggles (CAUTION: never look at the arc without appropriate eye protection). Preliminary calculations show that a carbon neck of 5 mm^3 volume, when evaporated over a spherical surface 10 cm in radius, will yield a carbon layer that is 40 nm thick.

Following carbon-coating, the vacuum chamber is slowly returned to ambient pressure, and the filters are removed and placed in their respective holders or in clean, marked Petri dishes for storage on a clean bench.

Transfer of the Sample to the EM Grid—

Transferring the collected particulates from the carbon-coated polycarbonate filter to an EM grid is accomplished in a clean room or on a class-100 clean bench. The transfer is made in a Jaffe wick washer, which is usually a glass Petri dish containing a substrate to support the EM grid/carbon-coated membrane filter combination. Solvent is added to a level to just wet the combination and cause gentle dissolution of the membrane with minimum loss or dislocation of the particulates, resulting in a membrane-free EM grid with particles embedded in the carbon film coating. The substrate support can be stainless steel mesh bridges, filter papers, urethane foams, or combinations of these.

The refined Jaffe wick washer is described as follows:

(1) The glass Petri dish (diameter, 10 cm; height, 1.5 cm) is made airtight by grinding the top edge of the bottom dish with the bottom of the cover dish, with water and Carborundum* powder (80 mesh); this creates a ground-glass seal (closer fit) and minimizes the need to refill the Petri dish with added solvent. (The usual glass Petri dish was found not to retain the solvent for long periods of time, and unless the wicking substrate is kept continuously wet, poor solubility of the membrane filter results, leading to a poor-quality EM grid).

(2) A combination of foam and a single sheet of 9-cm filter paper is used as the substrate support. A 3-cm x 3-cm x 0.6-cm piece of polyurethane foam (the packing in Polaroid film boxes) is cut and placed in the bottom dish. A 0.5-inch V-shaped notch is cut into the filter paper; the notch is oriented in line with the side of the foam, creating a well for adding solvent. Spectrographic-grade chloroform (solvent) is poured into the Petri dish through the notch until it is level with the top of the foam (also level with the paper). The foam will swell, and care is needed to avoid adding solvent above the filter paper.

(3) On top of the filter paper, pieces of 100-mesh stainless steel screen (0.6 cm x 0.6 cm) are placed, usually in two rows, to make several grid transfers at one time (for such uses as replicas), and to facilitate maintenance of proper identity of each transfer.

(4) A 3-mm section (usually midway between the center and edge) of the carbon-coated polycarbonate filter is cut in a rocking motion with a scalpel. The section may be a square, rectangle, or triangle, and should just cover the 3-mm EM grid.

(5) A section is laid carbon-side down on a 200-mesh carbon-coated EM grid. (Alternatively, Formvar-coated† grids or uncoated EM grids may be used. Here, the carbon coating on the polycarbonate filter forms the grid substrate.) Minor overlap or underlap of the grid by the filter section can be tolerated, since only the central 2-mm portion of the grid is scanned in the microscope. The EM grid and filter combination is picked up at the edges with the tweezers and carefully laid on the damp 100-mesh

* Carborundum is a registered trademark of the Carborundum Co., Carborundum Center, Niagara Falls, N.Y. 14302.

† Formvar is a registered trademark of the Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, Mo.

stainless steel screen. The EM grid-filter combination will immediately "wet out" and remain on the screen.

(6) Once all specimens are placed in the washer, more solvent is carefully added through the notch to maintain the liquid level so that it just touches the top of the paper filter. Raising the solvent level any higher may float the EM grid off the mesh or displace the polycarbonate filter section.

(7) The cover is placed in the washer and oriented in place over the specimen, and a map of the filter/grid/screen arrangement is made on the glass cover and in the logbook.

(8) Solvent (chloroform) is added periodically to maintain the level within the washer until the filter is completely dissolved by the wicking action (24 to 48 h).

(9) The temperature in the room must remain relatively constant to minimize condensation of solvent on the bottom of the cover and subsequent falling of solvent drops on the EM grid. Should day-night or other temperature differentials occur, solvent condensation on the under-surface of the cover can be minimized by placing the Jaffe washer at a slight tilt (three glass slides under one edge of the Petri dish parallel to the row of grids) to allow the condensation drops to flow toward the lower edge rather than fall on the EM grids. At temperatures lower than 20°C (68°F), the complete filter solution may take longer than 72 h.

(10) After the polymer is completely dissolved, the stainless steel mesh screen with the EM grid is picked up while wet and set on lens paper tacked to the bottom of a separate Petri dish. The EM grid is then lifted from and placed next to the screen to dry. When all traces of solvent have evaporated, the grid is stored in a grid box and identified by location and grid box in the logbook.

Figure A4 illustrates the Jaffe wick washer method; Figure A5 shows the washer. The foam/filter combination is currently preferred, as is use of a closely fitted (by means of the ground-glass seal) Petri dish.

Gold Coating—

An additional step will aid in subjectively evaluating the SAED pattern. This step is required for specimens from the upper Great Lakes area and for those of unknown origins. After the particulates on the filter are transferred to the EM grid, the grid is held to a glass slide with double-stick tape for gold-coating in the vacuum evaporator. Several EM grids may be taped to the glass slide with double-stick tape for gold-coating in the vacuum evaporator. For comparison, one-half of the EM grids may be coated and the other one-half not coated; recognition of the gold-coating is helpful in searching and x-ray analysis. Several EM grids may be taped to the glass slide for

coating at one time. Approximately 10 mm of 0.015-cm-diameter (0.006-inch) pure gold wire is placed in a tungsten basket (10 cm from the rotating table holding the EM grids) and evaporated onto the grid.

The thin gold-coating establishes an internal standard for SAED analysis. For some mineral species, an internal standard will clarify visual identification of the pattern of a fibrous particulate as being or not being an amphibole species (for example, minnesotaite as opposed to amosite). With experience, differentiation in SAED patterns can be observed. For samples of known geographic origins, gold-coating is optional, since the additional coating hinders observation and identification of small-diameter chrysotile fibers.

4. TEM Examination and Data Collection

Figure A10 shows a modern TEM with capabilities for elemental analysis with an EDS. The grid is observed in the TEM at magnifications of 250X and 1000X to determine its suitability for detailed study at higher magnification. The grid is rejected and a new grid used if: (1) the carbon film over a majority of the grid openings is damaged and not intact; (2) the specimen is dark due to incomplete dissolution of the polycarbonate filter; or (3) the particulate loading is too light (unless a blank) or too heavy with particle-particle interactions or overlaps.

TEM Analysis (Morphology, SAED, and X-Ray Analysis)—

The following guidelines are observed for consistency in the analytical protocol:

(1) Magnification at the fluorescent screen is determined by calibration with a diffraction-grating replica in the specimen holder.

(2) A field of view or "gate" is defined. On some microscopes, the central rectangular portion of the fluorescent screen, which is lifted for photographic purposes, is convenient to use. On others, a scribed circle or the entire circular screen may be used as the field of view. The area of the field of view must be accurately measurable.

(3) The grid opening is selected on a random basis.

(4) The analysis, morphology, and SAED are performed at a tilt angle of 0°.

(5) The recommended instrument settings are: accelerating voltage, 100 kV; beam current, 100 μA; film magnification, 20,000X (which is equivalent to 16,000X on the fluorescent screen for this instrument); and concentric circles of radii 1, 2, 3, and 4 cm on the fluorescent screen.

(6) The grid opening is measured at low magnification (about 1000X).

(7) Since asbestos fibers are found isolated as well as with each other or with other particles in varying arrangements, the fibrous particulates are characterized as asbestos structures:

Fiber (F) is a particle with an aspect ratio of 3:1 or greater with substantially parallel sides.

Bundle (B) is a particulate composed of fibers in a parallel arrangement, with each fiber closer than the diameter of one fiber.

Cluster (Cl) is a particulate with fibers in a random arrangement such that all fibers are intermixed and no single fiber is isolated from the group.

Matrix is a fiber or fibers with one end free and the other end embedded or hidden by a particulate.

Combinations of structures, such as matrix and cluster, matrix and bundle, or bundle and cluster, are categorized by the dominant fiber quality--cluster, bundle, and matrix.

(8) Counting rules for single fibers, which are illustrated in Figure A7 are as follows:

(a) Particulates meeting the definition of fiber are isolated by themselves. With this definition, edge view of flakes, fragments from cleavage planes, and scrolls, for example, may be counted as fibers.

(b) Count as single entities if separation is equal to or greater than the diameter of a single fiber.

(c) Count as single entities if three ends can be seen.

(d) Count as single entities if four ends can be seen.

(e) In general, fibers that touch or cross are counted separately.

(f) Two or more fibers are counted as a bundle if the distances between fibers are less than the diameter of a single fiber, or if the ends cannot be resolved.

(g) Fibrils attached longitudinally to a fiber are counted as part of the fiber and the size (width) is estimated based on the fiber-to-fibril relationship.

(h) A fiber partially hidden by grid wires (one or two sides of the grid opening) is counted, but labeled as an X-fiber (X-F) in the structure column. If the number of X-fibers is high enough to affect the size distribution (mass, etc.), a large-mesh EM grid should be used, such as 100 mesh (about 200 μm wide).

(9) Sizing rules for asbestos structures are:

(a) For fibers, widths and lengths are obtained by orienting the fibers to the inscribed circles on the fluorescent screen. Since estimates are within ±1 mm, small-diameter fibers have greater margins of error. Fibers less than 1 mm at the fluorescent screen magnification level are characterized as being 1 mm. A cylindrical shape is assumed for fibers. X-fibers are sized by measuring their entire visible portions in the grid opening.

(b) Bundles and clusters are sized by estimating their widths and lengths. The sum of individual diameters is used to obtain the total width, and an average length for the total length. A laminar-sheet shape is assumed, with the average diameter of the individual fiber as the thickness.

(c) Matrices are sized by adding the best estimates of individual fiber components. A laminar or sheet structure is assumed for volume calculation.

(10) The method of sizing is as follows:

(a) An asbestos structure is recognized, and its location in the rectangular "gate" relative to the sides, inscribed circles, and other particulates is memorized.

(b) The structure is moved to the center for SAED observation and sizing.

(c) Sizing is performed using the inscribed circles. If the structure, such as a fiber, extends beyond the rectangular gate (field of view), it is superimposed across the series of concentric circles (several times, if necessary) until the entire structure is measured.

(d) The structure is returned to its original location by recall of the location, and scanning is continued.

Analytical Procedure—

The analytical procedure is as follows:

(1) EM grid quality is assessed at 250X.

(2) Particulate loading is assessed at 1000X.

(3) A grid opening is selected at random, examined at 1000X, and sized.

(4) A series of parallel traverses is made across the grid opening at the film magnification of 20,000X. Starting at one corner, and using the tilting section of the fluorescent screen as a "gate" or "chute," the grid opening is traversed. Movement through the "gate" is not continuous, but rather is a stop/go motion. On reaching the end of one traverse, the image is moved the width of one "gate," and the traverse is reversed. These parallel traverses are made until the entire grid opening has been scanned.

(5) Asbestos structures are identified morphologically and counted as they enter the "gate."

(6) The asbestos structure is categorized as fiber (with or without X-) bundle, cluster, or matrix, and sized through use of the inscribed circles.

(7) The structure (individual fiber portion) is centered and focused, and the SAED pattern is obtained through use of the field-limiting aperture.

(a) SAED patterns from single fibers of asbestos minerals fall into distinct groups. The chrysotile asbestos pattern has characteristic streaks on layer lines other than the central line, and some streaking also on the central line. Spots of normal sharpness are present on the central layer line and on alternate lines (that is, 2nd, 4th etc.) The repeat distance between layer lines is about 0.53 nm.

(b) Amphibole asbestos fiber patterns show layer lines formed by very closely spaced dots, and have repeat distances between layer lines also of about 0.53 nm. Streaking in layer lines is occasionally present due to crystal structure defects.

(c) Transmission electron micrographs and SAED patterns obtained with asbestos standard samples should be used as guides to fiber identification. An example is the "Asbestos Fiber Atlas" (Mueller et al., 1975).

(8) From visual examination of the SAED pattern, the structure is classified as belonging to one of four categories: (1) chrysotile, (2) amphibole group (includes amosite, crocidolite, anthophyllite, tremolite, and actinolite), (3) ambiguous (incomplete spot patterns), or (4) no identification. SAED patterns cannot be inspected for some fibers. Reasons for the absence of a recognizable diffraction pattern include contamination of the fiber, interference from nearby particles, fibers that are too small or too thick, and nonsuitable orientation of the fiber. Some chrysotile fibers are destroyed in the electron beam, resulting in patterns that fade away within seconds of being formed. Some patterns are very faint and can be seen only under the binocular microscope. In general, the shortest available camera length must be used, and the objective lens current may need to be adjusted to give optimum pattern visibility for correct identification. A 20-cm camera length and a 10X binocular are recommended for inspecting the SAED pattern on the tilted screen.

(9) The specimen holder is tilted for optimum x-ray detection (40° tilt for the JEOL* 100C instrument's Tracor Northern† NS 880 analyzer and Kevex‡ detector). The categorized asbestos structure is maintained in its centered position for x-ray analysis by means of the Z-control.

(10) The spot size of the electron beam is reduced and stigmated to overlap the fiber. As an option for STEM instruments, the electron beam may be used in the spot mode and the x-ray analysis performed on a small area of the structure.

(11) The EDS is used to obtain a spectrum of the x-rays generated by the asbestos structure.

(12) The profile of the spectrum is compared with profiles obtained from asbestos standards; the best (closest) match identifies and categorizes the structure. The image of the spectrum may be photographed, or the peak heights (Na, Mg, Si, Ca, Fe) recorded for normalizing at a later time. No background spectra or constant acquisition time is required since the shape of the spectrum (profile) is the criteria. Acquisition of x-ray counts may be to a constant time; to a constant peak height for a selected element, such as silicon (1.74 keV); or just

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* JEOL (U.S.A.) Inc., 11 Dearborn Road, Peabody, Mass. 01960

† Tracor Northern Inc., 2551-T.W. Beltway Hwy., Middleton, Wis. 53562

‡ Kevex Corp., Chess Dr., Foster City, Calif. 94404

long enough to get an adequate idea of the profile of the spectra, and then aborted. Figure A11 illustrates spectra obtained from various asbestos standards and used as referenced profiles.

(13) The specimen holder is returned to 0° tilt to examine other asbestos structures.

(14) Scanning is continued until all structures are identified, measured, analyzed, and categorized in the grid opening.

(15) Additional grid openings are selected, scanned, and counted until either the total number of structures counted exceeds 100 per known area, or a minimum of 10 grid openings has been scanned, whichever is first.

(16) The TEM data should be recorded in a systematic form so that they can be processed rapidly. Sample information, instrument parameters, and the sequence of operations should be tabulated for ease in data reduction and subsequent reporting of results. Figure A12 shows an example of a data sheet used in Level II analysis.

Figure A9 illustrates the method of scanning a full-grid opening. The "field of view" method of counting, which is based on randomly selected fields of view, has been discontinued. Originally, the method was recommended for medium loading level on the filter (50 to 300 fibers per grid opening). However, if samples are collected at three different loading levels and the optimum is selected, this medium loading on the filter will not be used. Samples with grid openings containing 50 to 300 fibers may be used as laboratory fiber preparations or selected source samples, but in field samples, the particulate loading is usually of much higher concentration than the fiber. Filter loading is characterized by particulate concentration, not by fiber concentration.

EDS is relatively time-consuming, and becomes redundant if used as repetitive analysis for a confirmatory check on chrysotile fibers. Chrysotile identity by morphology and visual SAED analysis is not as controversial as amphibole identification and categorization.

The following rules are recommended for EDS analysis (Level II):

(1) For chrysotile structure identification, the first five are analyzed by EDS, then one out of every 10.

(2) For amphibole structure identification, the first 10 are analyzed by EDS, then one out of every 10.

(3) For amphibole structure identification and categorization, all confirmed amphiboles are analyzed by EDS.

(4) For ambiguous structure identification and categorization, all are analyzed by EDS.

Energy dispersive x-ray analysis as used in asbestos analysis is semiquantitative at best. X-ray analyzer manufacturers may claim quantitative results based on calibration standards and sophisticated computer software, but such claims are based on stoichiometric materials and extension of work with XRF instrumentation. Asbestos has a varying elemental composition. The electron beam in an EM is of varying size, and not all instruments are equipped to measure the beam current hitting the specimen. The size of the specimen has an effect on the x-ray output, and nearby materials may fluoresce and add to the overall x-ray signals being generated. Moreover, specimen tilting results in a loss of x-ray acquisition from particles hidden by grid wires or by other particles.

The only consistency in x-ray analysis is that the intensity of the output, within restrictions, is proportional to the mass, therefore providing the semiquantitative analytical possibility. Asbestos minerals have been found to have a characteristic profile, although not an exact duplicate of each other. For example, the Mg:Si ratio of chrysotile may vary from 5:10 to 10:10, averaging about 7:10. The ratio can be used to confirm the morphology and visual SAED analysis.

Table 1 illustrates the phenomena of variability with resemblance for some of the amphibole fibers. Peak heights and profile measurements were taken.

To aid in the visual perspective of the spectrum profile, the peak heights were normalized to a silicon value of 10, resulting in a five-number series that is relatively easy to visualize--as in the following examples:

chrysotile	~ 0-7-10-0-0
tremolite	~ 0-4-10-3-<1
crocidolite	~ 1-1-10-0-6
anthophyllite	~ 0-3-10-0-1
amosite	~ 0-2-10-0-7

These relationships are approximate, since chrysotile can vary from 0-5-10-0-0 to 0-10-10-0-0. However, for the others, the variation is only about one point, such that the profile (shape) of the five elements (Na, Mg, Si, Ca, Fe) is recognizable.

5. Data Reduction and Reporting of Results

Data Reduction—

From the data sheet, size measurements are converted to microns (16,000X screen magnification), mass of asbestos structure is calculated, and other characterizing parameters are calculated through use of a hand calculator or computer. (Appendix C, an example of a computer printout from Level II analysis, shows reduced data--that is, what was found on the specified number of grid openings or area examined.) These measurements are summarized and related to the volume of air sampled and the total effective filtration area

TABLE I. PROFILE COMPARISON OF ASBESTOS STANDARDS

Asbestos Type	Size, μ	Na	Mg	Si	Ca	Fe	Profile
Amosite (GF-38A)	0.19 x 1.44 (stigmate)		182	497		386	0-4-10-0-8
	0.19 x 0.75 (STEM)		186	528		387	0-4-10-0-7
	0.19 x 1.25		181	352		289	0-5-10-0-9
	0.19 x 0.88 (100 s)		226	870		674	0-3-10-0-8
	0.25 x 1.81 (100 s)		576	4207		3338	0-1-10-0-8
	0.12 x 1.56		253	2049		1515	0-1-10-0-7
	0.31 x 2.38		256	2127		1613	0-1-10-0-8
	0.19 x 1.56		276	1696		1116	0-2-10-0-7
	Repeat		477	2945		1959	0-2-10-0-7
Anthophyllite (AF-45)	0.56 x 2.38 (stigmate)		631	2577		349	0-2-10-0-1
	0.31 x 2.38 (stigmate)		640	1670		71	0-4-10-0-0
	0.31 x 5.19 (stigmate)		1064	3610		466	0-3-10-0-1
	0.19 x 1.56 (stigmate)		507	2191		309	0-2-10-0-1
	0.19 x 1.88 (stigmate)		787	2286		257	0-3-10-0-1
Crocidolite (CR-37)	0.19 x 0.81 (stigmate)	131	100	885		501	2-1-10-0-6
	0.06 x 0.50 (stigmate)	28	28	205		115	1-1-10-0-6
	0.06 x 0.69 (stigmate)	37	35	171		96	2-2-10-0-6
	0.12 x 1.00 (stigmate)	44	53	379		204	1-1-10-0-5
	Repeat (STEM)	70	64	612		333	1-1-10-0-5
	0.12 x 0.62 (stigmate)	56	65	479		260	1-1-10-0-5
	0.12 x 1.12 (stigmate)	53	56	326		166	2-2-10-0-5
	0.19 x 1.56 (stigmate)	78	83	735		421	1-1-10-0-6
	0.06 x 1.69 (stigmate)	45	48	290		159	2-2-10-0-6
	Repeat (STEM)	72	85	892		463	1-1-10-0-5
	Repeat (STEM)	35	42	373		237	1-1-10-0-6
	Repeat (STEM)	16	22	166		104	1-1-10-0-6
Tremolite (T-79)	0.38 x 2.19 (stigmate)		138	368	93		0-4-10-2-0
	0.38 x 2.19 (spot)		114	327	80		0-4-10-2-0
	0.25 x 1.75 (stigmate)		80	197	65		0-4-10-3-0
	0.25 x 1.75 (spot)		95	252	62		0-4-10-2-0
	Repeat (stigmate)		70	211	51		0-3-10-2-0
	(STEM-100 s)		376	1118	245		1-3-10-2-0
	(STEM-100 s)		135	364	72		0-4-10-2-0
	(STEM-100 s)		1454	4810	1235		0-3-10-3-0
	(STEM-100 s)		64	191	48		0-3-10-2-0
	(STEM-100 s)		1072	3114	882		0-3-10-3-0
	(STEM-40 s)		46	113	27		0-4-10-2-0
	(STEM-40 s)		123	333	94		0-4-10-3-0

(area of deposit). Size measurements of X-fibers may be doubled and noted, or kept as a separate category.

Fiber number concentration is calculated from the equation

$$\text{Fibers/m}^3 = \frac{\text{Total no. of fibers}}{\text{No. of EM fields}} \times \frac{\text{Total effective filter area, cm}^2}{\text{Area of an EM field, cm}^2} \times \frac{1}{\text{Volume of air sampled, m}^3}$$

The number of X-fibers, bundles, clusters, and matrices are calculated in a similar manner. X-fibers may be included with fibers if they are few in number. Similarly, their corresponding mass (from their size measurements) may be included.

Fiber mass for each type of asbestos (chrysotile or amphibole) in the sample is calculated by assuming that both chrysotiles and amphiboles have circular cross-sections (cylindrical shape) and that the width measurements are one diameter. The density of chrysotile is assumed to be 2.6 g/cm^3, and of amphiboles to be 3.0 g/cm^3. The individual mass is calculated from the equation

$$\text{Mass, } \mu\text{g} = \frac{\pi}{4} \times (\text{length, } \mu\text{m}) \times (\text{diameter, } \mu\text{m})^2 \times (\text{density, g/cm}^3) \times 10^{-6}$$

The total mass concentration of fibers for each type of asbestos is then calculated from the total mass of all the individual fibers of that type.

The individual masses of bundles, clusters, and matrices are calculated by assuming a laminar or sheet-like structure with an average thickness of the fiber make-up of the structure. Again, the density of chrysotile is assumed to be 2.6 g/cm^3, and of amphiboles to be 3.0 g/cm^3. The individual masses are calculated from the equation

$$\text{Mass, } \mu\text{g} = (\text{length, } \mu\text{m}) \times (\text{width, } \mu\text{m}) \times (\text{thickness, } \mu\text{m}) \times (\text{density, g/cm}^3) \times 10^{-6}$$

The total mass for each type of structure for each type of asbestos is the sum of all the individual masses.

Other characterizing parameters of the asbestos structures are: (1) length and width distribution of fibers, (2) aspect ratio distribution of fibers, and (3) relationships of fibers, bundles, clusters, and matrices.

Reporting of Results—

The data and their subsequent reduction are reported as summarized, or can be further reduced to present the interrelationships of the various characterizing parameters. Figure A13 is an example of the EM data report; Figure A14 is an example of the sample summary report.

The methodology can establish the limits of identity for unknown samples, act as a QC/QA method for Level I analysis, and satisfy most of the identification criteria for asbestos.

6. Quality Control/Quality Assurance

Sampling procedures will vary depending on the type of sample, objectives of the sampling, and time/cost factors. The primary goals of sampling are to obtain a representative sample at the location and time of sampling, and to maintain sample integrity. The sampling team will have written sampling procedures, and the field chief and/or designated individual will be responsible for all record-keeping (including sample identification, labeling, logging of data, site description, and meteorological conditions), pre- and post-collection checks, and continuous sample custody and sign-outs until the sample is delivered to the laboratory and transferred to the appropriate quality assurance officer (QAO). Verification of sampling times, flow rates, equipment calibration, and taking of field blanks will be checked and recorded in the field logbook.

Samples are turned over to the QAO for logging into a project logbook. Each sample is carefully examined for gross features, such as tears, breaks, and overall condition of container. The QAO registers the as-received sample number and other designated information, and assigns a simple internal code number that will accompany the sample through the preparation stage, grid transfer, grid analysis, data reduction, and reporting of results.

After being logged into the project logbook, the sample is transferred to the custody of the electron microscopy staff, where every precaution is taken to maintain sample integrity and to prevent contamination and loss of collected particulates. During storage and transport, the filters in their respective holders are maintained in a horizontal position at all times.

The sample logging, handling, and storing procedures ensure that all samples can be readily located and identified throughout the course of a program. The QAO has divisional responsibility for QC/QA activities, and must see that the laboratory maintains high standards. He must be aware of current standards of analysis, and must ensure that internal quality control standards, instrument calibration, and records of samples and completed analyses are kept for ease of later retrieval and use.

For quality control, internal laboratory blanks are analyzed at least once a week, which may or may not coincide with a sample batch blank. In addition, a magnification calibration of the EM using a carbon grating replica (2,160 lines per mm) is performed once a week. The results are recorded in an EM instrument log, along with other routine instrumental performance checks. All photographs, TEM, SEM, and STEM images are recorded in a photo log. These QC results are documented for inspection by the QAO.

SECTION 6

LEVEL III ASBESTOS ANALYSIS

DISCUSSION OF PROTOCOL

The Level III protocol is an extension of the Level II analysis procedures described in Section 5. This extension may be necessitated by the need for positive identification of the specific amphibole species in situations where (1) fundamental disagreements between parties involved in a litigation require further clarification; (2) for identification purposes, e.g., as causative agents in medical diagnosis or studies; (3) for quality control of Level II analysis in special situations, and/or; (4) for source samples whether as bulk material or bulk-air type where a legal judgment is anticipated.

Since an SAED pattern may be considered as a signature of the crystal structure of the diffracting crystal (mineral fiber or particulate), the mineral giving the pattern can be identified by comparison of measured and standard sets of d-spacings and interplanar angles (θ) from SAED patterns obtained in near-exact zone axis orientations. Such identification, however, may not be absolute without the provision of SAED patterns from more than one zone-axis orientation.

The Level III analysis is an objective, confirmatory-type analysis and consists of Level II analysis plus quantitative SAED analysis from two different near-exact zone-axis orientations on a selected number of fibers identified for detailed SAED analysis during the course of Level II analysis.

The Level III analytical procedure consists of locating the selected fibers contained in gold-coated grid openings (for internal calibration); photographing the fibers under bright-feld illumination; obtaining (by tilting) and recording two zone-axis SAED patterns from each selected fiber; and obtaining (recording and photographing) representative EDS spectra from the subject fiber.

The present Level III protocol is based on the following guidelines:

(1) Maintenance of procedural continuity so that results of Level II analytical effort will aid in conducting the Level III effort.

(2) Since detailed SAED analysis on all the fibers measured in Level II analysis is not possible due to time and cost restraints, a selection criterion is needed to assure representative analyses.

(3) The primary emphasis in Level III analysis is on the positive identification of the amphibole type.

(4) The present protocol is designed to allow greater flexibility and freedom of decision for the microscopist in determining the selection criteria since, due to practical constraints (position, orientation, contamination, etc.), all fibrous particulates may not be suitable for detailed SAED work.

(5) It is recommended that approximately 20% (at least 10%) of the fibers examined in Level II analysis be selected for Level III SAED analysis. Fibers which would be classified as "amphiboles" or "ambiguous" in Level II analysis should be more often included for Level III analysis as compared to those fibers which could be readily identified as "not asbestos." In cases where the majority of the fibers in Level II belong to a single, easily-identifiable species (e.g., chrysotile), fibers that are different should be more often selected for detailed Level III analysis. This flexibility in selection criteria will maximize the gain (meaningful information) from Level III effort beyond what would be achieved from the analysis of 10-20% randomly selected fibrous particulates.

(6) The electron microscope grids used in Level III analysis (also Level II if Level III is anticipated) should be finder grids so that location of fibers examined could be referenced for quantitative SAED and for future re-checking.

(7) Level III analysis should always be conducted by or under the close supervision of a professional electron microscopist knowledgeable in crystallography, SAED analysis, mineralogy, plus Level I and Level II asbestos analyses. If such expertise is not available in-house, an outside consultant should be retained.

(8) If enforcement proceedings and possible legal involvement may be part of the analytical procedure, the sample collection procedure entails additional record-keeping to maintain sample integrity. The field crew chief or a designated individual initiates, in addition to normal QC/QA activities, a chain-of-custody record. The sample is collected by the field team or by a representative of the adversary party in the presence of each other, and is sealed and signed for with the date and time. The designated individual acknowledges receipt of the collected sample. In transferring the sample, the designate signs a release of the sample in the presence of the new recipient, who notes the date and time, and signs for acceptance in the designate's presence. The chain of custody ensures that only responsible personnel have access to and control of the sample, thereby avoiding the possibility of

contamination before and after transport to the laboratory. At the laboratory, the QAO has first access to the sealed sample container, and signs for it after obtaining a signed release by the hand-carrier.

SUMMARY OF PROTOCOL

(1) An EM grid is prepared as directed in Level II analysis using finder or locator grids instead of regular 200-mesh grids.

(2) The particulate-loaded grid is then one-half or completely coated with a thin layer of gold.

(3) The gold-coated grid is placed in a tilt-rotation or a double-tilt specimen holder, and examined in the AEM or STEM.

(4) At low magnification the specimen grid is examined, and a grid opening is selected and identified for reference.

(5) Fibers identified for detailed Level III SAED work during Level II analysis, employing the selection criteria described under Level III guidelines, are now examined one at a time.

(6) A bright-field image of the fiber is taken at 0° tilt and at the magnification of analysis (20,000X).

(7) With the tilt-rotation or double-tilt combination, well-defined SAED patterns of two different zone-axis orientations are observed and photographed. The fiber location with respect to the edges of the grid opening or to other particulates may prevent more than one zone-axis orientation from being obtained for some fibers.

(8) X-ray elemental analysis is taken of the fiber after the SAED patterns. The EDS analysis also may be affected by proximity of the fiber to the edge of the grid opening or to other particles if tilting of the specimen is required for efficient use of the EDS. An image of the spectra is taken along with a record of the peak heights (the presence of grid peaks, such as Cu or Ni, as well as gold-coating may serve as markers).

(9) As explained earlier, due to time and cost considerations, at least 10% (preferably 20%) of the fibers examined in Level II are analyzed in Level III work.

(10) Those fibers whose EDS elemental analysis points to a possible amphibole identification are selected for SAED pattern indexing.

(11) Parameters of interest obtained form zone-axis SAED patterns are: the camera constant, CC (obtained from the gold ring); the diffraction spot spacing d_1 (along the slant vector), d_2 (along a row); the inter-row spacing, R; and the interplanar angle $\theta_{1,2}$. See Figure A18 for details.

(12) The reciprocal lattice values of the d-spacings d_1 and d_2 and the inter-row spacing (R) are converted into direct lattice spacings and then d_1, d_2, R, and $\theta_{1,2}$ are compared to those of standard amphibole species listed in JCPDS Powder Diffraction Files, values computed from lattice parameters and crystal structures, or SAED Standard Pattern File developed internally from known amphibole minerals regulated by EPA.

EQUIPMENT, FACILITIES, AND SUPPLIES

Essential items required for a Level III analysis are:

- A 100-kV AEM equipped with the fluorescent viewing screen inscribed with graduations of known radii to estimate the lengths and widths of fibrous particulates; or a modern 100-kV TEM equipped with an EDS. A scanning accessory as found in an STEM will increase the versatility and analytical capability for very small fibers or for fibers adjacent to other particulate matter. This microscope should also be equipped with the fluorescent viewing screen inscribed with graduations of known radii to estimate the lengths and widths of fibrous particulates.
- A specimen holder with tilt-rotation or double-tilt capability to obtain diffraction patterns at different zone-axis orientations.
- Darkroom facilities for developing negatives, making enlarged prints of patterns, and facilitating measurement of distances, spots, lines, and circles.
- A vacuum evaporator with a turntable for rotating specimens during coating, for such uses as carbon-coating polycarbonate filters, gold-coating EM grids, and preparing carbon-coated EM grids.
- An EM preparation room adjacent to the room housing the EM. This room should either be a clean-room facility, or contain a laminar-flow class-100 clean bench to minimize contamination during EM grid preparation. Filter handling and transfer to EM grids should be performed in a clean atmosphere. Laboratory blanks should be prepared and analyzed weekly to ensure quality of the work. In addition, a sample preparation room with a laminar-flow class-100 clean bench should be available for handling bulk-air

samples, ashing procedures, sedimentation, ultrasonification, filtration, and other prefilter activities.

- Several modifed Jaffe wick washers for dissolving membrane filters.
- Miscellaneous supplies and chemicals, such as membrane filters, EM grids, films, gold wire, chloroform, and carbon rods.
- Sample collection equipment, such as filter holders, sampling pumps, critical orifices, and tripods.

DESCRIPTION OF METHODOLOGY

A detailed discusson of the morphology, crystallography and chemistry of asbestos minerals, electron microscopy, and SAED analysis is outside the scope of the present protocol. Basic knowledge in these areas and an adequate level of comprehensive knowledge of TEM and SAED are prerequisites for the microscopists participating in asbestos analysis, especially at Level III stage.

Since Level III analysis is an extension of Level II analysis, common methodological details dealing with type of samples (source), sample collection and transport, sample preparation, TEM examination and data collection, data reduction and reporting of results, and quality control/quality assurance (QC/QA) program, which were discussed in detail in Section 5 (Level II Asbestos Analysis) will not be repeated here and the users are advised to refer to Section 5 for details in these areas. Differences, if any, between Level II and Level III protocols in common areas have been dealt with earlier under "Guidelines" and "Summary of Protocol."

The following provides brief descriptions of some of the essential areas of the Level III protocol that were not covered under Level II protocol.

1. Crystallography and Morphological Properties

Both crystallographic and morphological characteristics of asbestos minerals can help considerably in asbestos indentification and analysis. Chrysotile displays a unique narrow tubular morphology. The amphibole asbestos minerals have very similar morphologies--they are elongated along the z-axis (the chain direction) and generally lie with (100) planes approximately perpendicular to the electron beam. All varieties of amphiboles exhibit these Wadsley faults parallel to the length of the fiber.

Chrysotile possesses a cylindrical lattice which produces a unique SAED pattern. All the amphiboles, except anthophyllite, which is orthorhombic, have a monoclinic crystal structure. The amphiboles are double-chain silicates in which the fiber axis, z, has a repeat of 0.53 nm (inter-row spacing 'R' in real space, Figure A18). Since the other lattice parameters are also very similar, detailed zone-axis SAED analysis in more than one orientation is needed for positive identification. The non-asbestos forms of amphiboles have properties very similar to their asbestos counterparts, thus they must be distinquished from asbestos on the basis of morphology alone.

2. Chemical Properties--Elemental Analysis by EDS

Amphiboles are nonstochiometric minerals and often contain substitutional cations in varying amounts. Therefore, precise determination of their chemistry is difficult and positive identification based on chemistry alone is not reliable. This may be particularly pertinent when dealing with asbestos minerals present as minor constituents in mineral samples.

Elemental ratios, which are sometimes used to distinquish between asbestos types, often vary over wide ranges even in standard samples. The presence of gold coating, which would tend to preferentially absorb x-rays from lighter elements more than heavier elements, may make the situation even worse. In view of these ambiguities, and due to inherent practical difficulties in obtaining representative quantitative EDS elemental analyses from submicroscopic fibers, the present Level II and Level III protocols specify the use of only qualitative EDS spectra, which are often very valuable for screening purposes in the identification procedure. For example, in distinguishing between tremolite and actinolite type of amphibole, actinolite usually contains Fe, but tremolite does not.

3. Selected Area Electron Diffraction (SAED)

The method of obtaining an SAED pattern of a randomly oriented specimen is usually described in the EM instruction manual. The general directions for using the instrument to obtain and photograph SAED patterns are:

(1) Select the image magnification for the selected area.

(2) Bring the desired field of view to the center of the screen.

(3) Insert the appropriate field-limiting aperture (according to the desired field of view) into the beam path.

(4) Obtain the sharpest field-limiting aperture shadow.

(5) Confirm that the desired field of view is in the field-limiting aperture.

(6) Focus the specimen image; a photograph of the selected area image can be taken.

(7) Obtain the SAED pattern, remembering to retract the objective lens aperture from the beam path. The SAED pattern will be observed on the fluorescent screen.

(8) Select the desired camera length (the shorter the length, the better for SAED patterns of asbestos taken at high magnification).

(9) Focus the SAED pattern sharply. The beam stopper is used to intercept the bright center spot.

(10) For photography, the illumination is expanded (condenser reduced) after focusing the pattern, so that the pattern becomes barely visible (indistinct). A manual time exposure of approximately 20 to 30 s (maybe more depending on such factors as specimen and film) is required. The beam stopper can be left in place or removed from the beam path 1 to 2 s before closing the shutter. A double exposure of the specimen image and the SAED pattern can be taken if particle-to-particle spacing is adequate.

4. Use of Tilting to Acquire Exact Zone-Axis SAED Patterns

Determination of the Tilt Axis--

In the side-entry type electron microscopes, the instrument tilt axis is always fixed. However, the position of the tilt axis on the viewing screen shifts with magnification. Also, there is always an angular rotation between the image and the SAED pattern. It is highly desirable to know the location of the tilt axis on the viewing screen and its relationship vis-a-vis SAED pattern under the operating conditions to make effective use of specimen tilting for obtaining exact zone-axis orientations. The following steps can be used to locate the position of the tilt axis:

(1) A gold-coated EM grid with a standard asbestos mineral specimen on a polycarbonate replica film is placed in a tilt-rotation or double-tilt holder and inserted at 0° tilt into an aligned TEM set at 100 kV, 100 μA, 20,000X magnification, and 20-μm camera length operation.

(2) The image is focused on the fluorescent screen, which is at approximately 16,000X magnification.

(3) A circular hole in the polycarbonate replica is positioned in the center of the field of view.

(4) On tilting, the circular feature changes to an ellipse with the major axis unchanged, and indicates the position (direction) of tilt axis at that magnification. The minor axis shows the perpendicular direction to the tilt axis. A high tilt angle defines the tilt axis more accurately than a small tilt angle. Figure A15 illustrates the effect of tilt.

(5) A double-exposure photograph at 0° tilt and at some high tilt angle, such as 30°, is taken of the focused circular hole for reference.

Tilting—for zone-axis SAED Patterns--

Quantitative SAED requires knowledge of crystallography to obtain useful zone axis diffraction patterns from which precise measurements can be made for comparison with known asbestos standards on file. Thus the method of obtaining the visual SAED pattern of randomly oriented specimens, as in Level I and II analysis, is modified for quantitative SAED pattern analysis. It requires tilting of the specimen to align major crystallographic directions with the

electron beam. The zone axis is a line parallel to a set of intersecting crystal planes and nearly parallel to the electron beam. A zone-axis pattern thus gives regular repeat distances and even intensities of spots throughout the pattern.

Either a double-tilt or a tilt-rotation type specimen holder can be used for obtaining zone-axis patterns. A double-tilt holder is often preferred because tilt-rotation combination involves translational movement of the fiber during tilting, necessitating constant adjustment of the specimen-positioning controls to keep the specimen centered in the SAED aperture. On the other hand, it is much easier to obtain an accurate measure of the degree of tilt and perform systematic tilting with the tilt-rotation specimen holder. It is only necessary to rotate the specimen (fiber) until the tilt axis (as determined earlier) coincides with a major row of spots and then tilt until a major zone axis is parallel to the incident electron beam. Alternately, fiber axis of the fiber can be oriented either parallel or perpendicular to the tilt axis and then further tilting is used to obtain exact zone-axis orientations.

In order to avoid flip-flopping between image and diffraction modes while tilting, a recommended procedure is to defocus the diffraction pattern (the aperature becomes visible and the specimen/fiber can be seen in it) so that a double image of fiber in aperture can be seen with a poorly focused diffraction pattern. The movement of the fiber can then be tracked in relation to the spot pattern during tilting and kept centered in the SAED aperture by use of the specimen-positioning controls (knobs) of the microscope. Sometimes a larger aperture aids in the tracking-pattern recognition process.

An experienced electron microscopist can readily recognize the geometrical features like Kikuchi lines or Laue zones in the SAED pattern and use these to obtain the exact zone-axis SAED patterns. A detailed discussion of Kikuchi patterns and Laue zones and their utility in tilting experiments may be found in any standard text book on electron microscopy. Use of the double-tilt specimen holder is very helpful and less tedious in tilting experiments. However, all laboratories may not have both types of specimen holders available. A skilled microscopist can use either specimen holder without much difficulty. Experience and skill are more important factors in SAED analysis than the type of specimen holder used.

5. Characteristics of SAED Patterns Encountered in Asbestos Analysis

Successful application and exploitation of SAED analysis in asbestos analysis needs prior knowledge of the general appearance and distinguishing characteristics of other SAED patterns which are often encountered. The following discussion summarizes some of the observed SAED features of asbestos and other related minerals. This discussion is by no means comprehensive and assumes that the reader is familiar with general crystallography and the nomenclature pertaining to varous aspects of SAED patterns.

Minnesotaite and Stilpnomelane--

These iron-rich non-asbestos layer minerals are often encountered in asbestos analysis of specimens from certain geographic locations. Particulates of these minerals lie near their basal (001) planes. Stilpnomelane and

minnesotaite both possess large superlattices and their commonly observed SAED patterns are easily distinguishable from amphibole patterns. The spacing (in reciprocal space) is about half (for minnesotaite) or less than that for most amphiboles. These minerals can be readily distinguished in Level I or Level II analyses if a gold coating (optional) is applied to the specimen grids. A visual inspection of the number of rows of spots inside the (111) gold ring is sufficient to distinguish minnesotaite and stilpnomelane from amphiboles.

Chrysotile--

Due to the cylindrical lattice of chrysotile the SAED pattern is unique. The SAED pattern observed is symmetrical about the cylinder axis, x, and the spacing of the rows of spots is proportional to 1/a, where a is 0.53 nm. The most distinguishing features of the pattern are the flared spots of the type (130) which occur in the firt layer line. The flaring is due to the cylindrical lattice. A typical EDS spectra shows the presence of only Mg and Si (Figure A11).

Amphiboles--Systematic Absences, Twinning, and Double Diffraction--

The most commonly observed row of diffraction shots found in SAED patterns in amphiboles is in the y* or b* direction, representing the shortest reciprocal spacing between the spots (18.4 Å in real space). There are many strong zone axis orientations containing the y* row of spots. The lattice of amosite, crocidolite, tremolite, and actinolite is c-centered, and for such a lattice the h + k odd spots are absent along the y* or b* row. In practice, however, weak spots may be present in forbidden positions due to the presence of thin multiple twinning on (100), which cause streaking parallel to a*. Often, reciprocal nets from both twins are present in the same SAED pattern. In a twinned crystal, the number of important diffraction nets containing b* is doubled, leading to the observation that the diffraction patterns appear insensitive to tilt.

In some cases SAED patterns can contain spots from both twin individuals which overlap. However, not all the spots present in the composite SAED patterns are generated by the overlapping nets; some spots may be present because of double diffraction where a diffracted beam from one twin becomes the transmitted beam when it enters the other twin.

The purpose of the above discussion is to point out that although many complications exist in the analysis of SAED patterns, these can be overcome; in a good goniometric tilting stage most amphiboles can be identified by SAED analysis.

Amosite--

The nearest reciprocal lattice section to the (100) direct lattice plane in amosite is (301)* and it is also the most commonly observed section. Due to the presence of the thin (100) twins, this section closely resembles (100)*.

Typical EDS spectra from amosite fibers (Figure A11) show mainly Si and Fe with smaller amounts of Mg and Mn. Mn is frequently observed as a substitutional cation in amosite.

Crocidolite--

Most of the commonly observed patterns are asymmetrical and cannot be indexed easily. However, they all show rows of spots separated by a reciprocal repeat (R) corresponding to the fiber axis (0.53 nm).

The main elements observed in typical EDS analysis are Mg, Si, Ca, and Fe. Na, which is usually present in crocidolite, may not be detected in gold-coated specimens because of absorption, or because of overlapping secondary peaks from the copper grid.

Tremolite-Actinolite--

Temolite and actinolite show a variety of SAED patterns which have very similar appearances. In actinolite some of the Mg is replaced by Fe, with the result that interplanar d-spacings of actinolite are slightly larger than tremolite. In both tremolite and actinolite, the main elemental constituents are Mg, Si, and Ca. Actinolite also contains some Fe.

Anthophyllite--

Even though anthophyllite has an orthorhombic crystal structure, its commonly observed patterns are similar to the monoclinic amphiboles. Anthophyllite fibers dehydrate more easily in an electron beam and are, therefore, more difficult to study.

EDS elemental analysis shows the main constituents to be Si and Mg with a small amount of Fe.

6. Determination of Camera Constant and SAED Pattern Analysis

As mentioned earlier, a thin film of gold is evaporated on the specimen EM grid to obtain zone-axis SAED patterns superimposed with a ring pattern from the polycrystalline gold film. Since d-spacings corresponding to identifiable gold rings are known, these can be used as an internal standard in measuring unknown d-spacings on an SAED pattern from a fiber. The precision of measurement is as good as the quality of the photograph (or negative) and usually the measurements should be in the order of 0.1-0.2 mm with an angular tolerance of 0.5-1.5 degrees. The measurements can be made by several methods: manually with a ruler, with a mechanical aid, or a densitometer, etc. The patterns can be read directly on the developed negative or on an enlarged non-glossy print.

In practice, it is desirable to optimize the thickness of the gold film so that only one or two sharp rings are obtained on the superimposed SAED pattern. Thicker gold film would normally give multiple gold rings, but it will tend to mask weaker diffraction spots from the unknown fibrous particulates. Since the unknown d-spacings of most interst in asbestos analysis are those which lie closest to the transmitted beam, multiple gold rings are unnecessary on zone-axis SAED patterns.

7. Determination of Camera Constant Using Gold Rings

An average camera constant using multiple gold rings can be determined as explained below. However, in practice, in most cases determination of the

average camera constant is not necessary and thicker gold films are not desirable. The camera constant, CC, is 1/2 the diameter, D, of the rings times the interplanar spacing, d, of the ring being measured and is expressed as:

$$CC(mm\text{-}\text{Å}) = \frac{D(mm)}{2} \times d(\text{Å})$$

The value of d for each ring can be obtained from the JCPDS file.

(a) Measure the diameters (two perpendicular locations) of the gold rings in mm as precisely as possible (see Figure A16).

(b) Measure as many distinct rings as possible to minimize systematic errors.

(c) Example: if the measured values in mm are D_1, D_2, D_3, D_4, and D_5, these will represent, respectively, d-spacings of

$$\frac{4.079}{\sqrt{3}}, \frac{4.079}{2}, \frac{4.079}{\sqrt{8}}, \frac{4.079}{\sqrt{11}}, \text{ and } \frac{4.079}{\sqrt{12}} \text{ Å}$$

(d) The camera constants will be:

$$CC_1 = \frac{D_1}{2} \times \frac{4.079}{\sqrt{3}} = \frac{D_1}{2} \times 2.355$$

$$CC_2 = \frac{D_2}{2} \times \frac{4.079}{2} = \frac{D_2}{2} \times 2.04$$

$$CC_3 = \frac{D_3}{2} \times \frac{4.079}{\sqrt{8}} = \frac{D_3}{2} \times 1.442$$

$$CC_4 = \frac{D_4}{2} \times \frac{4.079}{\sqrt{11}} = \frac{D_4}{2} \times 1.23$$

$$CC_5 = \frac{D_5}{2} \times \frac{4.079}{\sqrt{12}} = \frac{D_5}{2} \times 1.178$$

(e) The camera constant for the SAED pattern is the average of CC_1, CC_2, CC_3, CC_4, and CC_5. Table 2 presents an example of camera-constant determination.

TABLE 2. DETERMINATION OF CAMERA CONSTANT (EXAMPLE)

Ring No.	D_i readings (mm)	Mean D_i (mm)	d-spacing, d_i (Å)	Camera constant $C_i = D_i/2 \times d_i$
1	23.0, 22.0	22.5	2.355	26.5
2	27.4, 27.6	27.5	2.04	28.0
3	37.8, 38.2	38.0	1.44	27.4
4	44.6, 45.4	45.0	1.23	27.7

Mean Value of Camera Constant $= \frac{\Sigma C_i}{n} = \frac{26.5 + 28.0 + 27.4 + 27.7}{4} = 27.4$ (mm-Å)

8. Measurement of d-Spacings and Interplanar Angles

The gold film, because of its small, randomly oriented crystallites, produces a ring pattern superimposed on the SAED pattern from the fibers. The diameters of the gold rings correspond to known values of d-spacings, and this provides an internal standard to correct for inherent uncertainties present due to variations in instrumental and/or operating conditions. Since the d-spacings of interest on SAED patterns are usually the ones that lie closest to the center spot (transmitted beam), a camera constant measured from the first gold ring in the direction of measurement of d-spacings will usually give better accuracy in computed spacings than the use of an average camera constant. This method will account for any distortions in the symmetry of the gold ring pattern. The zone-axis SAED pattern usually has several rows of spots within the circular pattern of the gold rings. These rows of spots contain information about the two sets of planes in the crystal structure and the angle between them. The following procedure outlines the steps necessary to obtain the distances between planes (d-spacings) and the corresponding interplanar angle, θ (see Figure A17):

(1) From the spot pattern, determine the row with spots most closely spaced, and designate this as a horizontal row. Draw a fine line to show the row through the origin, and designate this the zeroeth row. Draw fine lines to show the first and succeeding horizontal rows. For a few horizontal rows, measure the mean spacing between adjacent spots (or the minimum vector):

$$X_i = \frac{\text{Distance between spots m units apart}}{m}$$

where m is chosen as an optimum number to minimize measurement errors. The mean horizontal spot distance, X,

equals the summation of X_i divided by the number, n, of rows measured. The d-spacing in Å corresponding to this vector is the camera constant divided by X, and is labeled d_2. Table 3 presents an example of spot spacing measurement within a horizontal row.

(2) The perpendicular distance between two adjacent horizontal rows is similarly measured. This interrow spacing, Z, is the mean separation between horiziontal rows, and equals the distance between a number of rows divided by the number of spaces. This distance is an additional vector for comparison that coincides with the slant vector, d_1-spacing, when angle $\theta_{1,2}$ is 90°. The row-spacing (R) equals the camera constant divided by Z. Table 3 presents an example of perpendicular spacing between horizontal rows; Figure A17 illustrates spot and row spacing.

(3) To obtain the d_1-spacing and corresponding angle $\theta_{1,2}$, a perpendicular is drawn to the zeroeth horizontal row through the origin. A line is drawn to the first spot to the right of the perpendicular in the first row and extended through the succeeding rows. This line, called the slant vector, forms the acute angle $\theta_{1,2}$. The mean spacing, Y, between spots on the slant vector can be measured by dividing the maximum distance between spots by the number of spaces between them, or by calculating from the interrow spacing:

$$Y = \frac{R}{\sin \theta_{1,2}}$$

The d-spacing in Å corresponding to this vector is the camera constant, CC, divided by Y and labeled d_1.

$$d_1(\text{Å}) = \frac{CC \times \sin \theta_{1,2}}{R} = \frac{CC}{Y}$$

Figure A18 illustrates the relationship of d_1, d_2, $\theta_{1,2}$ and R. In some cases, the interplanar angle $\theta_{1,2}$ may be more than 90 degrees (not shown in Figure A18).

Summary of Data from Each SAED Pattern:

(a) The camera constant, CC, as determined from the gold rings, normalizes the distances on the SAED pattern regardless of such factors as magnification and tilting.

TABLE 3. DETERMINATION OF SPOT SPACINGS (EXAMPLES)

Reading	Separation (mm)	Units	Mean spacing, X_i (Å)
Spot spacing within a horizontal row, d_2:			
1	49	16	3.006
2	42.7	14	3.05
3	--	--	--
			3.028 = Mean

$$\text{d-spacing} = \frac{27.4}{3.028} = 9.05 \text{ Å}$$

Perpendicular spacing between horizontal rows, R:			
1	43	8	5.0375
--	--	--	--
--	--	--	--
			5.0375 = Mean

$$\text{d-spacing, R} = \frac{27.4}{5.0375} = 5.44 \text{ Å}$$

Note: It is preferable that the camera constant values used in computing d-spacings are measured from the first one or two gold ring diameters in the direction of d-spacing measurement.

(b) The parameters of interest are:

- d-spacing of spots in a horizontal row: $CC/X = d_2$
- d-spacing of spots in the slant vector: $CC/Y = d_1$
- angle $\theta_{1,2}$ formed between a horizontal row and slant vector
- d-spacing corresponding to row separation as an additional parameter of interest: $CC/Z = R$.

It should be noted that the use of camera constant in the form used here in calculating d_1, d_2, and R, which are measured in reciprocal space on SAED patterns, automatically converts the calculated numbers into real space spacings, which are then compared to those from a suitable standard file.

9. Identification of Unknown Fibers

Unknown d-spacings (d_1 and d_2), interrow spacing (R), and interplanar angles (θ) measured from zone-axis SAED patterns of unknown fibers are compared with corresponding known values tabulated in JCPDS powder diffraction files, or those computed using lattice parameters and crystal structures of candidate asbestos minerals, or with the values contained in an internally developed file from standard specimens of candidate minerals. Table 4 is an example of the IITRI standards file (Jones et al., 1981). Figures A19 to A22 are examples of zone-axis SAED patterns.

Unknowns are matched as closely as possible to the file parameters for positive identification. However, considerable care and competent judgment are required in Level III confirmatory analysis. For example, amphiboles are usually nonstochiometric minerals, and thus a perfect match may not be possible between the d-spacings and interplanar angles determined from unknown fibers and those available from standard minerals. JCPDS Powder Diffraction files do not list interplanar angles. Since amphiboles have low-symmetry crystal structures, tabulated values of d-spacings and interplanar angles would be extensive and very expensive to generate, and to get an accurate match may not be possible because these tables are derived assuming certain lattice parameters which may not be the same as those of the unknown fibers being analyzed. Given these inherent uncertainties, it would seem that use of internally developed SAED files consisting of several readily accessible orientations (by virtue of natural habit of amphibole fibers) from standard amphibole species could eliminate a lot of tedious unnecessary work and yet provide reliable data for comparison and identification of unknown fibers.

In practice, SAED analysis combined with qualitative EDS analysis may help resolve certain cases where a close match in d-spacings and interplanar angles is not possible. For difficult specimens or SAED patterns of controversial nature, a second opinion may be necessary, especially if a legal case is involved.

TABLE 4. COMPARISION OF d-SPACINGS FROM SAED FILE AND POWDER DIFFRACTION FILE (EXAMPLE)

		Internal Standard File Data				Powder Diffraction File Data (1975)		
Amphibole type	Zone axis	d_1 (Å)	d_2 (Å)	θ (deg)	Interrow spacing, R (Å)	d_1 (Å)	d_2 (Å)	File index no.
Amosite	[100]	5.3	9.14	90.0	5.3	5.22	9.20	17-725
	[30$\bar{1}$]	1.79	9.26	84.0	--	1.76	9.20	17-725
	[101]	4.88	9.23	74.0	5.17	4.84	9.20	17-725
	[$\bar{1}$01]	4.14	9.11	78.0	4.21	4.10	9.20	17-725
	[$\bar{3}$10]	5.22	5.13	95.0	--	5.22	5.12	17-725
Crocidolite	[100]	5.22	8.97	90.0	5.22	5.20	9.02	19-1061
	[101]	4.94	9.05	75.0	5.19	5.89	9.02	19-1061
	[$\bar{1}$10]	4.79	8.19	79.0	5.23	4.89	8.40	19-1061
	[30$\bar{1}$]	1.75	8.97	83.5	--	1.76	9.02	19-1061
	[$\bar{3}$10]	5.12	5.12	96.0	--	--	--	19-1061
Tremolite	[100]	5.04	9.03	90.0	--	5.07	8.98	13-437
	[101]	4.83	9.03	75.0	--	4.87	8.98	13-437
	[$\bar{2}$0$\bar{1}$]	2.59	8.97	80.5	--	2.59	8.98	13-437
	[30$\bar{1}$]	1.72	8.98	83.5	--	1.69	8.98	13-437
Anthophyllite	[100]	--	--	90.0	5.24	5.28	8.90	9-455
	[$\bar{1}$42]	4.56	4.56	60.0	--	4.50	4.50	9-455